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A WELCOME TO PROFESSOR ANTHONY DAVID SMITH ON WHOM THE TITLE "DOCTOR HONORIS CAUSA" WAS CONFERRED BY THE COUNCIL OF JÓZSEF ATTILA UNIVERSITY

We greet Professor ANTHONY DAVID SMITH on whom the title "Doctor Honoris Causa" was conferred by the Council of József Attila University (Szeged, Hungary) at the ceremonial meeting of the Council of József Attila University on 21st September, 1993. On this occasion, he presented a lecture entitled "Cholinesterase - from basic science to clinical practice". The complete text of his lecture is published in this issue.

ANTHONY DAVID SMITH was born in China in 1938, where his father spent several years as a missionary. During the Second World War, the family returned to England and ANTHONY DAVID SMITH was educated at Kingswood School in Bath; then, in 1959, he began his studies at Christ Church College in Oxford. He achieved first-class honours in Biochemistry in 1963, and then carried out research for a D. Phil. degree at the Department of Pharmacology under the guidance of Professor HERMAN BLASCHKO, Fellow of the Royal Society. After several years of successful research, they provided the first biochemical evidence of the exocytosis of hormonal substances from the adrenal medulla. The beginning of his scientific cooperation with Dr. ISTVÁN BENEDECZKY dates from this period, when he published his results in the same field in Nature. DAVID SMITH was appointed University Lecturer in Pharmacology and elected a Fellow of Christ Church College in 1971. In 1979, he was awarded the Gaddum Memorial Prize of the British Pharmacological Society for his discoveries on the mechanism of release of the sympathetic transmitter noradrenaline. In 1979, he introduced an antenatal test for spina bifida in early pregnancy that is used throughout the world. In 1984, he was appointed Professor of Pharmacology and Head of Department of Pharmacology at the University of Oxford, and in 1985, the Medical

Research Council appointed him Honorary Director of the Anatomical Neuropharmacology Unit. In 1987, Professor SMITH negotiated a research agreement with BRISTOL MYERS SQUIBB, which led to the donation of 20 million pounds to the University in order to provide a new building of 6000 m^2 for the Department of Pharmacology and funding for research projects on brain diseases for 12 years. The building was completed in 1991.

Professor SMITH's interests relate to Alzheimer disease, schizophrenia, and the control of movement and blood pressure by the brain. He is chairman or member of the board of a number of international scientific journals.

Professor SMITH has a wide range of international scientific connections including Hungarian scientists, such as Professors VIZI, KÁSA and BENEDECZKY (the last was the head of the Zoological Department at József Attila University from 1982 until his retirement in 1993). His co-director at the MRC Anatomical Neuropharmacology Unit is PÉTER SOMOGYI, also "Doctor Honoris Causa" of the József Attila University.

Professor SMITH has very intensively supported József Attila University: first of all, as chief editor, he regularly sends the outstanding journal Neuroscience free for ten years, he has presented a large number of scientific books to the Department of Zoology and he has also helped us with fine chemicals. As a continuation of the cooperation, KATALIN HALASY, lecturer at the Department of Zoology, has now spent altogether more than two years in Oxford.

Professor SMITH is a world-famous scientist, with several thousands of citations of his work, including those results which were achieved together with the earlier-mentioned Hungarian scientists. After taking into consideration his remarkable scientific activity and the enormous help that he provided to the Department of Zoology at our University, the University Committee decided unanimously to confer on him the title "Doctor Honoris Causa".

We wish to congratulate Professor SMITH on being awarded this title and wish him every further success in his scientific research.

K. HALASY

ACTA BIOL. SZEGED. 40, pp. 5-9. (1994)

CHOLINESTERASE - FROM BASIC SCIENCE TO CLINICAL PRACTICE

A speech on the occasion of the conferment of 'Doctor Honoris Causa' József Attila University, Szeged 21 September 1993

A. D. SMITH

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Rektor, members of the Council of the University, Ladies and Gentlemen: It is with great pleasure and with a sense of honour that I stand before you today.

I would like to tell you a little about the process of discovery in science as I have been fortunate to experience it. The topic I have chosen is: "Cholinesterase - from basic science to clinical practice". What I will try to show you is how experiments carried out to answer basic questions of science can lead, in time, to a contribution to the practice of medicine. The experiments on cholinesterase that I shall describe were not carried out with any practical application in mind; at the time they were done I was not even aware of the problem in clinical medicine to which they were eventually to provide a solution.

I would like to illustrate four of the many factors that contribute to the path of discovery:

first, the primacy of experimental observation over current dogma;
second, the dedication and determination of the young scientist;
third, curiosity, without which life in science is barren;
fourth, the chance encounter with a colleague.

First, let me illustrate how experimental observation can challenge current dogma. I expect that everyone here will have heard of the chemical transmitter acetylcholine, which is secreted from certain nerves in the brain and in the peripheral nervous system. Its best known role is as the transmitter at the junction between the motor nerve and the muscle fibre: it is the agent that causes contraction of the muscles in your legs and arms. In order to stop the muscle from going into a spasm it is obviously necessary to get rid of the acetylcholine as soon as the muscle has contracted: that is the role of cholinesterase, an enzyme which hydrolyses the acetylcholine to inactive products.

Cholinesterase exists in the body in two different forms: the form that destroys acetylcholine after it has been released from nerves is called acetylcholinesterase and is present in the membranes of the muscle and nerve. The second form is called non-specific cholinesterase and is present free in the blood plasma. No one knows the function of the cholinesterase in the blood, but it is known that it is in the blood because it is secreted from the liver. By the early 1970's it was established that the two forms of cholinesterase were separate proteins and we now know that they are coded

by genes on different chromosomes. A dogma developed that stated that the two forms of cholinesterase were not only different proteins but that the acetylcholinesterase of nerves and muscle was an enzyme that was permanently fixed to the cell membranes, in contrast to the soluble form of cholinesterase in the blood plasma. You can find this dogma in many textbooks of the time and, indeed, in textbooks today. How did this dogma arise? Perhaps it was because people assumed that because an established function of acetylcholinesterase was to destroy acetylcholine after it had acted, the enzyme that did so must be fixed to the cell membrane near the nerve ending from which the acetylcholine was released. In other words, there was no need to consider any additional or alternative role for acetylcholinesterase.

Just 20 years ago, in 1973, IAN CHUBB and I were trying to isolate the synaptic vesicles that store acetylcholine from the nerves that innervate the adrenal gland. In line with current dogma, we were using the enzyme acetylcholinesterase as a marker for the fragments of membranes of the nerves in the different fractions we obtained after centrifugation of an homogenate of the nerve. We were very surprised when we found that in fact only about half of the acetylcholinesterase was attached to the membranes, the rest was soluble in the homogenate. Because of the prevailing dogma, we assumed that the soluble acetylcholinesterase was some sort of artefact and that it had come off the membranes during the experiment. However, we did many experiments that convinced us that this was not the case: the soluble acetylcholinesterase was different from the membrane-bound enzyme in several respects.

What was the role of this soluble acetylcholinesterase? We assumed that it was soluble in the homogenate because it had been trapped inside a cell particle that was broken open during homogenisation. It seemed unlikely that it was anything to do with the destruction of acetylcholine and so we wondered whether it had another function. A few years earlier, LILIANA LUBINSKA in Warsaw had shown that acetylcholinesterase was one of the proteins that is rapidly transported along the nerve trunk from the cell body towards the nerve endings. It had been assumed that this transported acetylcholinesterase was attached to the membranes. We wondered whether the soluble form of acetylcholinesterase might in fact be being transported towards the nerve ending in order for it to be secreted from the nerve. We were, in fact, able to show that this is the case: acetylcholinesterase can be secreted from the nerve endings upon stimulation of the nerve. In other words, nerve cells not only secrete small molecules that are transmitters but also a large molecular weight protein.

Perhaps you can imagine the skepticism that greeted our findings. After all, we had challenged two accepted dogmas: first that acetylcholinesterase is invariably bound to membranes and second that nerve cells can only secrete small molecules. I am pleased to say that the experimental evidence finally overcame both these dogmas, but it was surprisingly difficult to convince some people!

Now I will illustrate the second factor on the path to discovery: the dedication and determination of the young scientist.

In this case, the young scientist in question is Hungarian: his name is PÉTER SOMOGYI and two years ago you honoured him with Doctor Honoris Causa. The story begins in 1970 with a visit for one year by ISTVÁN BENEDECZKY to my laboratory in Oxford. István is well known to all of you because he has just retired as your Professor of Zoology. István is a wonderful teacher and I was fortunate to learn much from him during his period in Oxford. Not long after he returned, he wrote to me about a young student who had first been taught by his wife Rózsa in school; she had spotted his talents, and then PÉTER SOMOGYI had worked with István on a project during his time as an undergraduate. István wrote that Péter would benefit from a period in another University. I was impressed by István's recommendation and I managed to find a small scholarship for Péter, who by then had already won a national prize in Hungary for an essay he had written on the mechanism of secretion from nerves.

We had just the project for Péter. István had taught him how to use the electron microscope and we wanted to find out where in the nerve cell the soluble form of acetylcholinesterase was located. One of the pioneers of the electron microscopic localization of acetylcholinesterase was PÉTER KÁSA of the Medical University here in Szeged and so I asked Péter if he would be able to learn the method from Kása before he came to Oxford. Péter did just that: he traveled from Budapest to Szeged each day to carry out experiments and, because he was a poor student, the only way he could travel was by hitch-hiking along the road. Now Péter is not one to waste any time: rather than spend hours waiting in Szeged while the samples were incubating, he carried the reagents with him in his rucksack and changed the solutions on the way back to Budapest. Of course, I knew nothing about this before Péter arrived in Oxford but I was tremendously impressed that he had already mastered the necessary technique before he had arrived. Within a short time he had done the critical experiments that gave a clue as to where soluble acetylcholinesterase is stored in nerves and how it could be secreted from them. Furthermore, before he left Oxford he had written an excellent paper describing the results, that was accepted without change in the Proceedings of the Royal Society.

Dedication and determination: these are two invaluable qualities for a scientist and Péter's discoveries led quite naturally to the next part of the story.

The third factor that contributes to the path of discovery is curiosity, something no scientist should be without. Now that we had shown that acetylcholinesterase was a secretory protein in the peripheral nervous system, I could at last satisfy my curiosity about a fact that had long puzzled me. The cerebrospinal fluid that baths the brain and spinal cord contains much less protein that does blood plasma but the dogma of the time was that this protein was derived by filtration from the blood. You may recall that the cholinesterase of blood plasma is the non-specific form. On the other hand, the cholinesterase activity in cerebrospinal fluid is largely due to the presence of acetylcholinesterase and the amount of the non-specific cholinesterase is very small. These facts were puzzling: could it be, I wondered, that the acetylcholinesterase present in cerebrospinal fluid is in fact derived from them nerves in the brain and that these nerves secrete acetylcholinesterase just as we had found nerves outside the brain

to do? There was only one way to satisfy my curiosity about this question: do an experiment. This I did with SALLY GOODMAN and IAN CHUBB: we were able to show that stimulation of sensory nerves in the periphery, a procedure that was known to activate certain pathways in the brain, led to an increase in the concentration of acetylcholinesterase in the cerebrospinal fluid. Shortly afterwards SUSAN GREENFIELD joined me and showed that electrical stimulation applied to certain parts of the brain itself also led to an increase in the concentration of acetylcholinesterase in the cerebrospinal fluid. We were then able to demonstrate secretion of acetylcholinesterase from isolated brain slices, so establishing acetylcholinesterase as a neurosecretory protein in the brain. Incidentally, although this is another story, the curiosity of SUSAN GREENFIELD has led her to demonstrate that the secretory form of acetylcholinesterase has unusual modulatory actions on nerve cells in the brain that are totally unrelated to its ability to destroy acetylcholine.

So I come to the fourth and final example of factors that contribute to discovery: the chance encounter with a colleague. There must always a place for chance in scientific discovery, be it a chance observation in the laboratory or the chance remark of a colleague and we should keep our minds open for such occasions.

The year was 1978: my wife and I were entertaining for dinner a Swedish doctor, HUGO LAGERCRANTZ, whom, 7 years earlier, I had examined for his MD thesis in Stockholm. He had moved into clinical medicine and was telling us the sad story of how, within the previous 3 months they had terminated the pregnancies of two women who were suspected of carrying a fetus with spina bifida only to find that the fetus was perfectly normal. Now in spina bifida as I expect you know, the spinal canal does not close up properly and so cerebrospinal fluid leaks from the spinal cord into the amniotic fluid. I asked him how they had diagnosed spina bifida and he told me that it was by measuring alpha-fetoprotein in the amniotic fluid. I had never heard of this procedure, but I said: 'Why on earth do you use a liver protein to diagnose spina bifida, when what you want is a protein that is present in normal cerebrospinal fluid. We have just such a protein: it is acetylcholinesterase'. He agreed with me and within a few weeks he had returned to Oxford with some samples of amniotic fluid which I analyzed blindly. I found the neurosecretory form of acetylcholinesterase to be present in some of the samples; in every case it turned out that the samples with acetylcholinesterase were from pregnancies where the fetus had spina bifida. Within a few months we had collaborated with NICK WALD and his colleagues in Oxford and had been able to establish a new test for spina bifida in early pregnancy.

Now a diagnostic test is only of value if it is better than the previous test. In fact, the previous alpha-fetoprotein test was very good in comparison with tests for other diseases. Nevertheless, it had a false positive rate of about 1%. What does this mean in human terms?

In the United Kingdom there are about 500,000 births each year and the incidence of spina bifida is about 0.5%; so, about 2,500 children each year could be born with spina bifida. For this reason, there is a national screening programme to detect spina bifida and to offer the mother the opportunity to terminate the pregnancy.

The detection rate of the screening programme is very good indeed, being 99%, but the problem is that, with a false-positive rate of 1% as many as 5,000 perfectly normal pregnancies each year could be terminated.

What, then is the false-positive rate of the acetylcholinesterase test? There have been two international studies and one very detailed study in Denmark: the most recent conclusion is that the acetylcholinesterase test has a false-positive rate one tenth of that of the alpha fetoprotein test. In human terms that means that, because of the use of the acetylcholinesterase test, up to 4,500 normal children are born each year in Britain who might otherwise have not lived. Although the prevalence of spina bifida is not high in other countries as in Britain, it is likely that the introduction of the acetylcholinesterase test in 1979 has saved the lives of tens of thousands of unborn children across the world.

So, my friends, I hope you will now understand the title of my talk. When we began these studies on cholinesterase in 1973

we were led by simple curiosity,
aided by the dedication of a young Hungarian student,
given courage to challenge accepted dogma,
and fortunate to hear from a colleague of a clinical problem that our findings could go a long way towards solving.

Finally, I should like to take this opportunity, Rektor, to thank the University most warmly for the honour they have bestowed upon me. As a token of my gratitude and that of my Department, we would like to offer to the József Attila University a scholarship so that you can send one of your students to Oxford University for a period of one year. The student would be able to work in one of the laboratories in the Department of Pharmacology or in its associated Medical Research Council Anatomical Neuropharmacology Unit. We would cover the costs of travel from Szeged and provide funds for living expenses in Oxford and for travel to a scientific meeting in Britain. I hope, Rektor, that this scholarship will help to build up even closer relations between our two Universities and to foster the pursuit of natural science.

Thank you very much.

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A WELCOME TO PROFESSOR CHARLES SUSANNE, ON WHOM THE TITLE "DOCTOR HONORIS CAUSA" WAS CONFERRED BY THE COUNCIL OF JÓZSEF ATTILA UNIVERSITY

On 9th September 1994, the title "Doctor Honoris Causa" was conferred on Professor CHARLES SUSANNE, head of the Laboratory of Anthropogenetics and Ecotechniques at the Vrije University in Brussels, coordinator of TEMPUS, in recognition of his great efforts to develop the Biological Departments at JATE, and especially his help in promoting the recognition of Hungarian anthropology and ecology outside the borders of Hungary. On the occasion of the conferral ceremony, Professor SUSANNE delivered a lecture entitled "Universities have to promote long-term politics: anthropology and ecotechniques as test cases". The text of this paper is published in full in this issue.

Professor CHARLES SUSANNE was born on 13th November, 1943, in Belgium. He was awarded his B.Sc. in 1966 and his Ph.D. in 1969, both in anthropology at the Université Libre de Bruxelles (ULB). After graduating, he joined the staff of the Vrije Universiteit Brussel (VUB), where he was associate professor between 1971 and 1977. Since 1978 he has been full professor at the VUB and in the next 10 years he was also an associate professor at the ULB, where he received the title of professor in 1987. He was visiting professor at University College London for 2 years. In 1989, he was elected Dean of the Faculty of Sciences at the VUB for 3 years.

Professor SUSANNE is leader of the Human Genetics Unit at the VUB, holder of the Unesco-Cousteau Chair, Ecotechnie, a Member of the Belgian Academy of Overseas Sciences, and a director of TEMPUS and Erasmus programs, including "Sciences", "Biology" and "Ecotechniques".

Professor CHARLES SUSANNE started his scientific work as an anthropologist. His main interest have included biology, especially demography and the genetics of human populations. Professor SUSANNE is one of the leading experts in this field of research. His scientific activities involve a wide range of anthropological, genetic and environmental sciences, including polyfactorial human genetics, the application of multivariate analysis in genetic studies, the role of genetic versus environmental influences on morphological characteristics, the development of asthmatic children, environmentally-induced genetic damage, the heritability of morphological characters, the interpretation of chromosome aberrations and the biometry of migrants. He recently focused his research on environmental problems and has contributed greatly to an outline of the paradigm of a modern human ecology.

Professor SUSANNE's scientific productivity is best illustrated by his more than 230 scientific papers, books and book-chapters. The majority of his articles have been published in high-standard international journals. He has been invited to more than 60 congresses and conferences to present plenary lectures, keypapers or seminars.

His recognition by the scientific community is indicated by the fact that he has been elected to offices on different international scientific and educational bodies, he has been President of the European Anthropological Association and the European Association of Human Ecology, Vice-president of the European Centre of Human Ecology, Director of the NATO Advanced Institute, a member of many scientific councils and boards, and a referee of 11 journals. He has been editor, vice-editor or editorial board member of 14 international journals.

Besides his research work and university teaching, Professor SUSANNE is also an outstanding organizer. He has organized 48 congresses and meetings and has been coordinator of different TEMPUS and Erasmus projects. He has many links with Hungary and Szeged. He organized the International Anthropological Meeting in the Department of Anthropology at JATE University in Szeged. As coordinator of TEMPUS projects "Biology" and "Ecotechniques", he provided numerous possibilities to our students and staff members to travel to European universities. His organizing activity contributed to the introduction of a postgraduate course in our university, which will give a European diploma in Ecotechniques to the more than 50 postgraduate students attending the course each year.

We warmly congratulate Professor SUSANNE on the award of this honorary title, and we all wish him good health, energy and activity to achieve further outstanding scientific results in the interest of mankind.

GY. L. FARKAS

ACTA BIOL. SZEGED. 40, pp. 13-18. (1994)

UNIVERSITIES HAVE TO PROMOTE LONG-TERM POLITICS: ANTHROPOLOGY AND ECOTECHNIQUES AS TEST CASES

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(Received: September 9, 1994)

As you know, I have many friends here in Szeged, in your departments of anthropology, ecology, and biology in general. When you asked me to say a few words, I felt embarrassed. Which topic to choice without disappointing some friends? I do not know if I will succeed, but I thought that one common denominator of this friendship was that all of us were dealing with studies of "long-term effects" and that all of us were confronted with problems relating to the structure of our teaching at least at an M.Sc. or Ph.D. level. Therefore, I will focus on these two topics and on the challenges linked to these aspects.

We often hear, and I believe it, that biology will become the science of the 21st century, and that the new biological techniques can change human evolution profoundly. It is true indeed that some modern techniques are very near to the science fiction of 20-30 years ago, and they are addressing us, or some of us. I do not want to speak here about in vitro fertilization, which will always remain a marginal way to procreate. I want to focus on the better prevention of disease,

the better treatment of disease,
the better detection of genetic disease,
the better treatment of genetic disease, possibly through genetic engineering,
the almost perfect possibility of contraception,
the choice of the sex of the child,
the choice of some other traits of the child,
the possibility of cloning,
the techniques of individual behaviour control,
the techniques of collective behaviour control, and
the techniques of efficient eugenism.

The problem is no longer how human beings will evolve, but who will choose how we will evolve and what control we can have over this choice.

We have never been in a situation where we could influence human beings, our society and our future so much. It is more than bioethics, it is global ethics, a part of human biology and anthropology.

Anthropologists are perhaps best placed to make this link between philosophy and science, because we can explain

human evolution and its limits,
the definition of Homo and its myths,
the structure of societies and its manipulations,
the human variation and its nationalistic abuses,
and the influence of sexuality and procreation and its phantasms.

These new challenges are in fact linked to the ethical problems of the definition of a person. Which "sacrality" to give to human life? Human beings have primarily an undetermined nature. Man is free enough to die for his freedom; seeing the good, he can choose the worse. His "humanitas" lies in its freedom, in his nature not to have a nature, in his capacity to fight over each code one would like to impose on him.

A new challenge for each of us probably also lies in the European political situation, where the European political movements were implicated in the past in the opposition between communism and liberalism. Today, sociodemocrats and/or liberals lack their past enemies, but are in fact not victorious; they are suffering from the breakdown of communism because somewhere these "enemies" were also the "allies".

A political vacuum has been created in East and West, and this vacuum has been filled up in many countries by nationalism. It is the concept of the general interest which is put in doubt. If the different human groups and the different cultures may not or even must not communicate with each other, and may not or even must not be mixed, if cultures must be pure and each reference to common values is only tyranny, then there is no choice: it is the way back to the romantic view of communities viscerally closed on themselves, it is the incapacity to surpass the atavic singularities to communicate with each other, and it is the opposition between particularism and universalism.

Human biology can again play an important role here because we know that a large part of human variability is in fact an intrapopulational variation. We know today how to interpret human variation: for instance, for the variance of enzymatic polymorphisms, 86% of the differences lie between individuals of the same population, 7% between populations of the same race and 7% between racial groups. For mtDNA also, most of the variability (90%) is attributable to differences between individuals in the same geographical race and only 10% to interracial differences (MELNICK et al., 1992).

It is no longer possible to justify aggression or simply discrimination on the basis of scientific arguments. But we know that xenophobia is still present, and that nationalism and nationalistic aggression are still part of our actuality.

In biology, it would be impossible in such a short time to mention the different challenges in all subfields of biology from molecular biology to ecology, from genetics to physiology, from geology to botany, and so on. Let me merely cite as an example the challenge of linking anthropology to human genetics and molecular genetics.

New challenges are indeed linked to the new discoveries in primatology (chromosomal, genetic and molecular data) and to the new discoveries of molecular anthropology (in terms of DNA analysis for taxonomic and phylogenetic purposes and in terms of mitochondrial DNA).

Studies at a biochemical level have confirmed the absence of a sharp division between humans and other primates.

The "molecular clock" has been constantly used and updated in recent years. Indeed, if one assumes that the differences between evolving lineages accumulate at a constant rate, than the fossil record can be used to calibrate a molecular clock of genetic distances between living species. There is now good agreement between the fossil and the molecular evidence of our ancestors, and about when the lines leading to modern humans, chimpanzees and gorillas began to separate.

Mitochondrial DNA has been used too. Differences in the genetic code of nucleus and mitochondrion are linked to their independent origin; moreover, mitochondrial genes lack the introns and the long parts of DNA without function. Mitochondrial DNA (mtDNA), with only about 16500 bases, has been completely analyzed in humans. Other helpful properties are their quick evolution (because there are no repair enzymes, mutations can not be corrected and mtDNA accumulates genetic changes at about 10 times the rate of nuclear DNA), their occasional crossing of their barriers between species, and of course their mainly matrilineal inheritance. It passes down the female line, because sperm provides almost no cytoplasm (and hence no mitochondria) to the fertilized egg. The matrilineal inheritance has led to publicity about the search for "Eve". The problem turned out to be a failure of understanding.

In fact, all these biochemical studies, but also the discovery of fossils on the one hand and studies of other animals (their behaviour, their anatomy and their biochemistry) on the other have confirmed the absence of uniqueness of our species; we are no longer on the pedestal on which we have wanted to place ourselves. This line of research has been among the most significant in anthropology. "The essence of evolutionary theory is not that all species are the same, but that they are all produced by the same biological processes and by the mechanism of natural selection. Now that the biological and evolutionary character of the hominids has been established, the main task facing paleoanthropologists is perhaps less to show that humans are not unique, for all species are unique, but to show how that uniqueness can be the product of processes that are themselves general to all living matter. We should recognize that we are a unique species, but also that we are just another unique species." (R.FOLEY, 1987).

In population genetics too, we are far away from an analysis of only blood groups and protein polymorphism: the knowledge of some polymorphism is becoming extremely precise at a molecular level and DNA technology is opening up new ways.

For instance, study of the highly polymorphic HLA is leading to new possibilities. Further, it is now possible to study a polymorphism of the Y chromosome. Indeed, the SRY male determining region has been discovered in a 35 kilobase segment near the end of the short arm of the Y chromosome and the gene

responsible for maleness has recently been found, the testis determining factor (TDF). It is known to translocate on the X chromosome and it has been used in experimentation to transform the sex of female embryonic mice for instance.

At a DNA level, we are in a period of molecular revolution in biomedical research: gene cutting, splicing, mapping, cloning and sequencing is ongoing. Initiatives are being taken to sequence the entire human genome. The use of these data in biological anthropology is only starting, but is already bringing unique perspectives (DEVOR, 1992):

restriction fragment length polymorphism,
DNA sequencing,
repetitive DNA,
hypervariable DNA,
mitochondrial DNA.

Perspectives exist for the study of new polymorphisms by anthropologists, but there is also a need for molecular biologists to understand the variation of the genome between individuals, between populations and between related species. In fact, they need some anthropological interpretation. The new DNA analysis does not change the philosophy of anthropological research; it permits only examination of

the genetic variability of the genome in more detail;
more genes (not only blood groups and some proteins);
the molecular basis of some morphological adaptations.

Ecotechniques are in fact related somewhat to anthropology and to human ecology because this is more than the study of the relationship as concerns humans and nature: this connection is not univocal, as humans are not only adapted to their environment, but also react to and alter it, freeing themselves from nature itself. During human evolution, the relation between humans and environment has undergone change and humans have become emancipated from nature by way of culture and technology.

Today, human beings are no longer considered the centre of the world: the cosmos is becoming the centre, which has to be defended against human beings. The biosphere is receiving an intrinsic value, higher than the value of the species Homo sapiens. A crisis is being created and the carrying capacity of the biosphere is forcing us to find a new relationship between man and nature. Human evolution is a history of symbiosis, control and later the domestication of nature; it is also a history of the violation of nature exacerbated by the exponential development of technologies and population densities.

Human beings are today obliged to take measures in favour of future generations, and to think in terms of long-term effects. Today, we are much more dependent on collective wisdom and ideological (or political) decisions. This is a result of our failing to consider human beings as forming part of the global ecosystem.

In anthropology, we regard man too often as an island outside nature; we have perhaps a tendency to think of him as being above nature because he developed original qualities and because he succeeded in the conquest of nature. In fact, we have

to consider anthropology in terms of ecology, and to place anthropology inside human ecology. Man is not opposed to nature; he is autonomous but dependent on it. We must give ecological thought to anthropology.

Ecology and the problems of Seveso, Bhopal, Chernobil, acid rain, the glass house effect, etc. crystallize the anxieties, and integrate technical and political problems. Ecology is evolving from the "ecological niche" studies to the biosphere problems.

The protection of nature is today synonymous with the protection of humanity: it is in short-term politics a protection of the quality of life, but in fact in long-term perspectives a protection of human life itself.

All these aspects influence our teaching of biology. The problem, but perhaps the advantage, of our teaching is that we must keep a broad basis; biologists will continue to be generalists, but we must avoid being specialists in nothing. A holistic approach must be kept at the beginning of the studies (in the graduate studies), where we must receive a very serious background of the different biological subdisciplines. This is also related to the job market, where employment in academic positions is diminishing: this means again the importance of acquiring overall training, even in non-biological areas, in order to expand employment opportunities beyond traditional biology. At a B.Sc. level, we do not have to fear to say as Socrates did "I know only one thing: that I know nothing". It is typical of biology, of science in general, of a spirit of science in any way, to find fewer certitudes than doubts, and fewer answers than interrogations. To become free of an illusion of comprehension is in fact a first step to knowledge.

Of course, at the M.Sc. and Ph.D. levels our students need appropriate training which will involve specialized teaching and research in one of the subfields of biology.

In other words, we must take our responsibilities to offer to our students a good B.Sc. level with a rather large biological background, with a not too narrow specialization, but offering to our M.Sc. and Ph.D. students a large network of expertise.

At the M.Sc. and Ph.D. levels we must also be honest with ourselves and confess that we can not offer the whole range of biology to our students: indeed, we have to be modest about the expertise our own home university can offer and we must be ready to help our students:

1. to choose the correct university for the topic in which they wish to specialize (not what we want to impose);

2. to choose the correct experts in each subfield: It is therefore important to promote: a network, the mobility of staff and students, joint projects.

Within TEMPUS biology, we took the initiative of a European Ph.D. in biology, and we hope to continue in this way.

Our strategy to maintain our academic freedom must be internationalization and must be networking.

Let us not fear envisaging ethical debates in our studies. A modern society can not exist without educating individuals to use their freedom of thought maximally, and without educating people to be continually critical, to respect rational laws, but to reject manipulations. We will not escape the biology of the 21st century and its possible risks; let us prepare ourselves to explain these risks, this education being the only way to preserve democracy.

Sartre stated that "L'Homme est obligé a chaque instant d'inventer l'Homme". I would say biologists are obliged to invent biology continuously. We are obliged to engage ourselves in the new debates, and to accept the corollary confrontation with others.

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IN MEMORIAM
DR PÉTER BERETZK
(on the occasion of the centenary of his birth in 1894)

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The region in which we live and work is more than simply a habitat of wildlife: it is a place where biologists gather their impressions. There is no exact ecological terminology that truly expresses the beauty of the scenery and birds, or can interpret human friendship, freedom and spirit, i.e. all that can be regarded as a real value in life. What we have learnt about these values is recognized through subjective experience: etymologically speaking this is expressed by the Latin word "subiectum". LORENZ was the first to stress the mistaken view that, epistemiologically, it is a failure to believe: by rejecting or denying subjective impressions, we can be objective.

There lived in Szeged a naturalist who founded a kind of "lake school": he gathered pupils and students around him and carried out his teaching and educational work in the open air there, and was an example as concerns how to pursue a naturalist's life.

This man, PÉTER BERETZK was born on 23 October, 1984 in Szeged. His father was a minister of Reformed Church. PÉTER BERETZK began his studies as a medical student in Kolozsvár, Transylvania, but the First World War put an end to his learning and as a medical volunteer he served in the field. Later, in 1918-20, he continued his studies and graduated as a gynecologist in Budapest. For a time he worked as a clinical assistant and then for 43 years was on the staff of the Hungarian Railways, finally resigning as directing senior surgeon.

PÉTER BERETZK inherited the universal desire of the old-time surgeon for a deep and overall knowledge of nature. He first came to the environs of Fehér-tó ("White Lake") in Szeged as a hunter. The lake at that time was the second largest natron lake after Fertő. As we can read in his diary, he was a great hunter, but years later he appears as an ardent bird-watcher and collector. From 1937 on, we do not find in his diary headings such as "Hunting"; instead, the contents reflect a man who recorded all details and observations. When he was a child, books by OTTÓ HERMAN had opened up for him the world of birds and later, as a hunter, he enthusiastically collected the nidatory and migratory birds at Fehér-tó. He did the preparation work himself and then sent the items in his collection to be identified by MIKLÓS VASVÁRI and JENŐ GRESCHIK in the Hungarian Ornithological Center.

After the exploration by KÁROLY LAKATOS to the world of Fehér-tó (before the turn of the century, today almost forgotten), it was PÉTER BERETZK, who revisited and described this unique avifauna. It was extremely interesting and important as a hatching area. In the remaining, small patches of ancient natron lake, avocets (*Recurvirostra avosetta*) were regular visitors for hatching. The number of mating pairs varied: regularly 20-25, but in 1946 for example about 100. Stilt birds (*Himantopus himantopus*) regularly visited this area (4-5 mating). Astonishingly large numbers of plovers (*Charadrius alexandrinus*) and terns (*Sterna albifrons*) nested there.

When a fish lake was founded there the uniformity and calmness of the ancient fauna was disturbed. New birds came which preferred undisturbed deep clear water. The variety of bird species observed widened, due to the reedy marshes, the bulrushes and the willowtrees. However, birds building their nests on the salt marshes became rarer, and the struggle between the "greens" and the husbandry collectives and lobbies has subsequently been a constant feature. PÉTER BERETZK observed that, besides the birds nesting there, the real and unsurpassed value of Fehér-tó is provided by the regular visits of rare migratory birds in certain seasons of the year. The most characteristic species of Fehér-tó are the mud-walkers: *Numenius, Charadrius, Totanus, Tringa, Limosa* and *Vanellus*. At the time of migration, tens of thousands of them appear on the smooth banks and on the muddy bottom of the dried-up lake. This tiny region gives a home to a wonderful and rich living world. The first man, to scientifically explore and describe the life of this area of sand and yellow soil of the Great Hungarian Plain was PÉTER BERETZK.

His connection with the town museum dated back to 1936. This was the year when he first placed here his collection of 123 prepared birds. In 1951, the collection, then numbering 900 pieces, was finally donated to the museum (see: CSIZMAZIA and GASKÓ: BERETZK PÉTER örökletéte. Móra Ferenc Múzeum Évkönyve 1984/85-I. (Donation by PÉTER BERETZK. Annals of Móra Ferenc Museum, 1984-85, Vol. I.)).

The museum opened its first (and to date by far the most popular and most frequently visited) exhibition under the title "The life of Fehér-tó". It is a pity that we can now only cherish its proud memory.

The name of PÉTER BERETZK is closely connected with the region he protected and explored. The citizens of Szeged, as well as people lowing and esteeming nature throughout Hungary regard the names of PÉTER BERETZK and Fehér-tó as one inseparable concept.

It is interesting that this fame is due not to the number of scientific papers he published in Hungary and abroad, but to the several hundred articles printed on the pages of daily newspapers (primarily the Szeged "Délmagyarország"). ISTVÁN HOMOKI-NAGY shot his firs film, "Vadvízország", here, and the great success of the film added to the recognition and popularity of the region.

Of course, at that time the "land of marshy tracts" was already slowly changing into a "land of regulated waters", of fish-lakes. Accordingly, PÉTER BERETZK and ISTVÁN GYÖRFFY (a university professor in botany) proposed to put the lake (at least partly) under protection. Law IV of 1935 made it possible for the town to declare 280 acres of the ancient alkaline marsh a protected area. In the following year, the Ministry of Agriculture sanctioned the town resolution and increased the area of protected land to 350 acres. Thus. the first Hungarian bird reserve was founded here.

The biological importance of the lake was described by PÉTER BERETZK in the following manner:

1. After Lake Fertő, this is the greatest natron lake in the country.
2. Along the River Tisza, it is nearest to the route of migratory birds.
3. The flora and fauna are extremely rich and characteristic.
4. With the development of other cultures (fishery and shrubbery), the species richness has markedly increased.
5. This allows a great variety of special biological research.

PÉTER BERETZK was an ardent photographer of nature as well. As a photographer, he was not a "hunter of trophies" (like so many of today's photographers), but did his job of gathering documents. His photos (taken with his favourite Leica camera) are of cultural and historical value: documents of changes in the life of the alkaline marshland, and of the evolution of the birdlife. He donated his enormous and valuable photo and slide collection to Móra Ferenc Museum. József Attila University in Szeged, the local Teacher's Training College, the Natural History Museum in Budapest, and the Ornithological Institute, all keep preparations made by him.

His closest friend was the ornithologist ANDRÁS KEVE. Every second month, they together walked around the lake, and registered the newcomers. ANDRÁS KEVE contributed considerably to the scientific success, and to the publication of papers by his friend. In 1945, PÉTER BERETZK became a distinguished member of the Hungarian Institute of Ornithology. He was a member of the Agra Academy of Zoology. The Nature Protection Society of Southern Finland appointed him a foreign member. In 1948, Szeged University honoured him with a professorship for his researches and results in exploring the bird life of the Hungarian alkaline marshes. He became a Candidate of Biological Sciences. In 1955, he was asked by the National Office of Nature Protection to fulfill the duties of scientific supervisor of the Fehér-tó region. In 1964, he became an extraordinary university professor. In the same year, he was

awarded the highest State Prize by the government. As a member of the Tisza-Research Team of the Hungarian Academy, he took part in expeditions, exploring the bird life along the river.

For 6 years, he was President of Szeged Section of the Hungarian Biological Society, the Honorary Life Member. For 10 years, he worked as President of the Biology Section of TIT (Association for the Advancement of Science) in Csongrád County. He initiated here the foundation of the section of Friends of Ornithology and Nature Protection (after his death, the Section took his name). He was Vice-president of the Dugonics Society.

PÉTER BERETZK corresponded widely with numerous Hungarian and foreign specialists. Hungarian and foreign ornithologists frequently enjoyed his hospitality when visiting the region. He died on 9 July, 1973 and is buried in the woodland.

His bibliography was compiled by GYŐZŐ CSONGOR on his 60th birthday; a complete bibliography of his publications was printed by BÉLA JAKAB. The total number of his various papers is 410, but some articles are to be found on forgotten pages of newspapers. According to ANDRÁS KEVE, the bibliography of his publications is somewhat irregular, since it is not common to include articles published in daily newspapers and magazines. However, these articles by PÉTER BERETZK are not only enjoyable to read, but also contain valuable scientific data on the fauna, ecology, etc. Seventy per cent (291 articles) of his publications fall into this category.

I consider that writings of this nature in a scientific-popular vein are of outstanding importance, since they contributed to the preservation of the bird-world of Fehér-tó under very difficult conditions, and this living museum with its unique avifauna continues to survive.

PÉTER BERETZK was a happy man. His work was widely known and recognized , and for a naturalist this is real happiness. As an ornithologist, his career was brilliant. He was a true-born teacher and by his example we have learnt how to love our everyday work and to perform it with belief, gladly and confidently. I am lucky to have been a disciple of PÉTER BERETZK. I can still wander along the pathways of Fehér-tó: in the small hut beside the lake, in the watch-tower of PÉTER BERETZK, I can carry on teaching new generations in his spirit. Dozens of his former students all over the country and here in Szeged, at he University, continue his research and nature protecting work, and remember the Master on the centenary of his birth.

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IN MEMORIAM DOZ. DR. ANDOR HORVÁTH

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(Eingegaben: 20. Jan. 1993)

Vor zwanzig Jahren verstarb eine hervorragende Persönlichkeit der ungarischen Malakologie, Dozent ANDOR HORVÁTH. Unser, die Hochachtung von Generationen geniessender "BANDI BÁCSI" (Onkel BANDI), an den sich seine Studenten und Kollegen mit fachlichen oder auch persönlichen Problemen vertrauensvoll um Rat wenden konnten, hat uns im Februar 1972 für immer verlassen. Er dessen Tätigkeit am treffendsten von Prof. AMBRUS ÁBRAHÁM wie folgt charakterisiert wurde: "Er war ein bekannter und anerkannter Molluskenforscher, insbesondere der Gastropoden, darüber hinaus war er ein so grosser Kenner vieler Tiergattungen, wie man ihn unter den ungarischen Zoologen, die gleichzeitig mit ihm das Tierreich zu ergründen suchten, kaum finden konnte." Diese Einschätzung ist kein Einzelfall unter den seit seinem Tode verlautbarten bzw. veröffentlichten fachlichen Beurteilungen.

ANDOR HORVÁTH erblickte am 5. November 1913 in Szabadka das Licht der Welt. Sein Vater war bis zu seiner Deaktivierung im Jahre 1913 Oberst der Ungarischen Wehrmacht, Oberkriegskommissar der Heeresverwaltung. Entsprechend den Diensteinteilungsorten des Vaters besuchte er in verschiedenen Städten die Schule. So die Grundschule in Budapest, Kaposvár und Pécs, die Gymnasien in Pécs, Budapest und in Szeged (z.B. das Gábor Klausál-Gymnasium). Von 1931 bis 1936 absolviert er die Universität in Szeged.

Nach Beendigung der Universität war er stellungslos arbeitete als Stundenlohnarbeiter in einem Militär-Lebensmittellager und von 1939 bis September 1940 war er unbesoldeter Assistent im Institut für spezielle Zoologie an der Universität Szeged. Im Jahre 1940 promovierte er sich auf dem Gebiet der speziellen Zoologie, für Allgemeine Zoologie und Allgemeine Geographie. Von 1940 bis 1943 wirkte er als Gymnasialprofessor in Kassa. Vom november 1943 bis 1951 war er zunächst in dem von Prof. BÉLA FARKAS geleiteten Institut für Zoologische Systematik, und nach dessen Ausscheiden in dem unter der Leitung von Prof. AMBRUS ÁBRAHÁM stehenden Institut für Allgemeine Zoologie und Biologie tätig. 1951 wird er wissenschaftlicher Assistent und erwirbt 1955 den Qualifikationsgrad "Kandidat der Biologischen Wissenschaften", bleibt aber dennoch - wohl wegen seiner Abstammung und seiner tiefen Religiosität - Universitäts "Adjunkt" (Oberassistent). Erst im August 1963 erfolgt die Ernennung zum Dozenten. Von 1954 bis 1967 ist er dann in dem unter

Leitung von Prof. GÁBOR KOLOSVÁRY errichteten Institut für Zoologische Systematik tätig, erhält aber auch nach dem Ableben von Prof. KOLOSVÁRY im Jahre 1967 keinen Lehrstuhl. Bis zu seinem Tode arbeitete er in dem von Prof. LÁSZLÓ MÓCZÁR geleiteten Institut für Zoologische Anatomie und spezielle Zoologie.

Während seiner Laufbahn unterrichtete er verschiedene theoretische Fachgebiete: Zoologische Systematik, Zoogeographie, Biologie, Sammeltechnik und Ökologie.

Nach dem Tode von ADORJÁN KESSELYÁK unterrichtete er von 1951 bis 1952 an der Hochschule für Lehrerbildung spezielle Zoologie, Anatomie, Parasitologie und leitete Fachlaboratoriumsübungen für Diplomanden. Er war Leiter der Geländeübungen in spezieller Zoologie. Er hielt mehrere Spezialkolloquien in Zoo-Ökologie. Seine gewaltigen Wandgemälde über die Tierwelt holarktischer Regionen verstauben heute im Keller. Sein bedeutendstes Spezialkolloquium, mit dem er die fachliche Nachfolge sicherte, war die Malakologie. Der Verfasser dieses Beitrages hat dieses Spezialkolloquium noch zwei Jahre nach Beendigung seines eigenen Studienganges fortgesetzt.

Auf die Erziehung des wissenschaftlichen Nachwuchses hat ANDOR HORVÁTH stets grösstes Gewicht gelegt. Diesem Umstand ist es zu verdanken, dass er auf seinem Fachgebiet, der Malakologie, die meisten Studenten er hatte. Sein erster Schüler war LÁSZLÓ IMRE, der die Erschliessung der östlichen Karpathen durchführte. Er fiel im 2. Weltkrieg. Seine sich mit rezentem Material beschäftigenden Schüler sind der Fachinspizient GYULA KOVÁCS, der Aussenstellen-Mitarbeiter am Munkácsy-Museum zu Békéscsaba, ANDOR RICHNOVSZKY an der Lehrerbildungsanstalt zu Baja, KÁROLY BÁBA an der Gyula Juhász Pädagogische Hochschule in Szeged und seine jüngsten Schüler MIKLÓS SZEKERES (SZBK, Szeged) sowie ERZSÉBET HORNUNG (JATE, Szeged).

Vier seiner Schüler beschäftigen sich mit fossilem Material: SÁNDOR ANTALFI (SZOTE, Szeged), MIKLÓS SZÓNOKY (JATE, Szeged), der Lehrstuhlinhaber Lajos Erdélyi (JATE, Szeged). An einem neurophysiologischen Thema arbeitet MIHÁLY MUCSI im Ölindustrielles Laboratorium (Szeged-Algyő).

ANDOR HORVÁTH war auch ein ausgezeichneter Kenner der marinen Mollusken. In seinen Spezialkolloquien unterrichtete u.a. die Systematik und Ökologie der Meeres-Weichtiere.

Nach 1957 leitete er den Studentenzirkel des Lehrstuhls. Über 30 Studenten erreichten unter seiner Anleitung den Doktorgrad der Universität. Wiederholt nahm er auch an der Arbeit des Wissenschaftlichen Qualifizierungs-Ausschusses teil.

Seine Vorlesungen hielt er ohne Konzept. Selbst bei zwei durch eine Semesterpause getrennte Unterrichtsgegenstände rekapitulierte er in der ersten Stunde des zweiten Semesters den letzten Satz der vorangegangenen letzten Stunde, um dann seine Lektion von hier fortzusetzen. Auf die während seiner Vorlesungen oder Übungen gestellte Fragen der Hörerschaft reagierte er stets geduldig und beleuchtete die Probleme vielseitig. Im Rahmen der Geländeübungen konnte man ihm kein noch so kleines Insekt zeigen, dessen lateinischen Namen er nicht gekannt hätte und von dessen Lebensweise er nicht ausführlich berichten konnte. Besonders gewissenhaft beschäftigte er sich mit seinen Diplomanden und mit den Hörern seiner Spezialkolloquien. Die vorgeschriebenen zweistündigen Übungen er streckten sich nicht selten auf vier bis fünf Stunden. Er konnte seinen Schülern zum väterlichen Freund werden. Die einzelnen fachlichen Übungen waren häufig ein wahres Erlebnis. Typisch für die Prüfungsabnahme war seine Hilfsbereitschaft. Er machte auch mit den Unbegabtesten, den nur geringe Fortschritte aufweisenden Studenten, keine Ausnahme. In seiner humanitären Art galt allen seine Hilfe - ohne Vorurteile.

Die Hauptcharakteristika seiner Persönlichkeit waren Bescheiden scheit, Aufrichtigkeit, Bedachtsamkeit und Hilfsbereitschaft. In wissenschaftlichen Diskussionen bediente er sich anstatt der damals üblichen heftigen, impulsiven Angriffe ausschliesslich der überlegten, geistigen Argumente. Er hatte ein ausgezeignetes Gedächtnis. In einer schlaflosen Nacht z.B. schrieb er - wie er erzählte - die lateinischen Namen aller ihm bekannten Arten zusammen: dreitausend an der Zahl.

Er war auch ein vorzüglicher Kenner der Ideologie seiner Zeit, obwohl er nicht mit ihr einverstanden war. Seine prinzipientreuen Diskussionspartner beschwichtigte er nicht selten mit den Argumenten der Hauptideologen dieser Zeit. Er war bekannt für seine aufrichtigen, treffenden Aussprüche. Einer der Professoren fragte ihn einmal besorgt: "Herr HORVÁTH, was geschieht mit dem Institut, wenn ich fortgehe? " Die Antwort war : "Herr Professor, wer kümmert sich nach dem Essen schon um den Löffel?" Oder: "Es macht nichts, wenn der Mensch angeschlagen, erschöpft ist. Er darf nur nicht vertrottelt sein!" Und mit der gesellschaftlicher Kritik: "Je leerer die Schnecke innen, um so lauter ist sie".

Sein wissenschaftliches Schaffen begann er mit einer Arbeit: "Formvarienten der Schnecken aus Szeged und Umgebung und ihre Bedeutung." In ihr wiederlegt er anhand zahlreicher Messungen seine Modelltheorie, die er eindeutig den Umwelteinflüssen zuordnete. Er kommt zu der Feststellung, dass bei den aus unmittelbarer Nähe gesammelten Schneckenindividuen grössere Abweichungen bestehen können, als zwischen den weiter voneinander entfert stammenden Exemplaren der gleichen Arten. Aufgrund der Gestalt kann bei Schnecken eine zoogeographische Einteilung nicht erfolgen (HORVÁTH, 1940).

Später entfaltet er sein vielseitiges Wirken auf mehreren Gebieten: Sammeln und Aufarbeiten der Fauna aus der Umgebung von Kassa (HORVÁTH, 1944). Hier in Kassa beginnt und in Szeged beendet er seine Arbeit über die Unterschiede der ungarischen Physa-Gattungen (HORVÁTH, 1950). Den grössten Teil seiner Arbeit machte die Aufarbeitung der rezenten und fossilen Mollusken der Ungarischen Tiefebene (Alföld) aus. Hiermit wurde er zum geistigen Erben der Zielstellungen der von Kogutovicz ins Leben gerufenen Alföld-Forschungskommission und setzte die von seinem Lehrmeister, MIHÁLY ROTARIDES, begonnene Arbeit."Die Schneckenfauna des Lösz, verglichen mit der heutigen Fauna mit besonderer Berücksichtigung der Löszbestände der Umgebung von Szeged" (1931) fort. Mit seiner ersten Unterschungen in dieser Richtung ("Aufarbeitung der Molluskenfauna der Theiss" HORVÁTH, 1943) ist er als der erste Theiss-Forscher zu betrachten. Mit Recht wird er 1957 Gründungsmitglied des von Prof. KOLOSVÁRY geleiteten, von der Akademie der Wissenschaften finanziell unterstützten Theissforschungs-Arbeitsausschusses. Weitere Arbeiten von ihm in Verbindung mit der Theiss erschienen 1957, 1958, 1962 und 1966, sowie posthum die aufgrund einer vorliegenden Skizze von seinem Schüler (BÁBA, 1972) in Druck gegebene Arbeit über Wassermollusken bzw. Schnecken der Theiss, ihres Wellenraumes und ihrer toten Arme.

Seine die Fauna des "Alföld" erschliessende Tätigkeit erstreckte sich neben der Theiss auch auf die Bearbeitung der Mollusken von Seen und Mooren der Ungarischen Tiefebene. Als erster studierte er die Mollusken des Weissen Sees (Fehértó) bei Szeged (1950), nahm die Erschliessung des trockengelegten Ur-Moores bei Kiskunhalas in Angriff (1953) und berichtete in Verbindung mit der Entwässerung der Moore des "Alföld" (Veresegyháza, Ócsa, Haláp, Bátorliget) über die Änderungstendenzen (1954). In Kenntnis des Molluskenmaterials der vom Staatlichen Geologischen Institut im südlichen Teil des Donau-Theiss-Zwischenstromgebietes angestellten umfangreichen Pleistozän-Erschliessungen wies er darauf hin, dass jene Moore, an denen (damals) noch keine menschlichen Eingriffe erfolgt waren, Reliktcharakter besitzen und teils den Zustand vor der Entwässerung wiederspiegeln bzw. teils Eiszeitcharakter bewahrt haben.

Er deckte die Sukzessionsgraduation der Veränderung der Moore bis zur Vernatronisierung und der totalen Austrocknung auf.

Hervorzuheben ist, dass A. HORVÁTH mit seinen Arbeiten - entgegen der faunistischen Vorstellungen von LAJOS SOÓS - ein Vertreter der von Rotarides begonnenen und seinerseits weiterentwickelten ökologischen Faunen-Untersuchung war, mit der er unter Mitwirkung seiner Schüler eine eigene Schule gegründet hat.

Anhand der Sammlungen von DEZSŐ LUKÁCS nahm er die Aufarbeitung des Schneckenmaterials des Djesna (Nebenfluss des Dnjepr) in Angriff (1951) und kam im Anschluss an seine erste Arbeit zu der Feststellung, dass bei Schnecken die

Veränderung der Körpergrösse, der Schalenlänge und -dicke, des Schliessapparates und der Flachheit der Schale im Vergleich zu den Exemplaren aus dem Dnjestr, der Theiss und der Sajó mit der Fliessgeschwindigkeit, mit der Strömungsstärke zusammenhängen bzw. wechselt.

Er hat auch bei der Erschliessung unserer Gebirgsgegenden mitgewirkt. So im Rahmen einer hydrobiologischen Arbeitsgruppe (ÁBRAHÁM, BENDE, MEGYERI) an der Erforschung der Molluskenfauna der Quellensysteme des Bükk-Gebirges, des Wassereinzugsgebiets um Putnok, im Bán-Tal, der Szilvás-Quelle und im Bereich des südwestlichen Bükk-Gebirges (1952, 1954, 1956 a, b).

Er legte den Grundstein zur Kenntnis der Mollusken des Börzsöny-Gebirges (1956). Angeregt durch seine Studien in unseren Gebirgsgegenden nahm Hochschulprofessor DEZSŐ LUKÁCS die Erforschung der in den Thermalgewässern und Quellen von Eger und Umgebung lebenden Schnecken sowie deren Adaptation an die abiotischen bzw. hydrochemischen Faktoren in Angriff. Ebenso auf Anregung von HORVÁTH machte sich ANTAL GEBHARDT an die malakologische Erforschung der bergwelt von Zselic und Mecsek.

A. HORVÁTH veröffentlicht posthum eine Monographie von Prof. JÁNOS WAGNER (WAGNER, 1952) : "Die Raublung-Schneckengattungen Daudebardia, Testacella und Poiretia. Eine systematische, geographische, ökologische und entwicklungsgeschichtliche Studie." Akad. Verlag Budapest. 1-259.

Auf Einladung verbrachte er längere Zeit in der Meeresforschungs-Station Split, wo er die Molluskensammlung aus der Adria aufarbeitete (1963). Einen Artikel veröffentlichte er über eigene und mit seinem Schüler eingeholte Sammlungen aus der Adria (HORVÁTH und BÁBA, 1967). Er revidierte das rezente Molluskenmaterial der Sammlung des Ferenc Móra-Museums in Szeged, deren wissenschaftsgeschichtlicher Wert darin besteht,dass sie auch die Clausalidae-Sammlungen von BRANCSIK vom Balkan beinhaltet.

Er veröffentlichte auch die Molluskensammlung des Ferenc Móra-Museums aus dem Schwarzen Meer (gesammelt VON MAGDA FERENCZ, DÁNIEL GÁL, JÁNOS ZSOLT und ANTAL KORMÁNYOS, 1963).

Seine vornehmlich marine Schnecken enthaltende Privatsammlung wurde gegen Entgelt der Zoologischen Sammlung des Ungarischen Nationalmuseums übereignet. dort aber nicht in die Bestandliste aufgenommen.

1950 gab er ein Fachgutachten über die industrielle Nutzung der Unio tumidus-Schnecken des Donauarmes bei Soroksár (als Hemdenknöpfe) ab. Diese Verwertung

der Schnecken wurde aber nach einigen Jahren infolge Auflösung des Betriebes eingestellt.

Den bedeutendsten teil seines Schaffens bildet die Synthese des ungarischen Pleistozän aufgrund der Molluskenfauna. Das Material dazu lieferten die von 1950 in Angriff genommenen Forschungsbohrungen seitens des Staatlichen Geologischen Instituts Ungarns, die sich von Szentes bis Baja, bis zur Löszwand bei Paks erstreckten und deren geologische Profile von MIHÁLY MIHALTZ gefertigt wurden. Diese Forschungsergebnisse bildeten das Thema seiner Kandidatendissertation (1954). Die Resultate seiner Forschungen wurden 1957 auf dem Madrider, 1958 auf dem Mexikoer und 1959 auf dem Warschauer Geologenkongress vorgetragen und anlässlich des Madrider Kongresses (1957) bzw. des Mexikoer Kongresses (1958) publiziert.

Die Veröffentlichung der Ergebnisse seiner Bohrungen bei Felsőszentiván vermochte er nicht mehr zu beenden (HORVÁTH 1962, 1963, 1964, 1965, 1966 I-V.).

Er erschloss die Molluskenfauna vor der Regulierung des Flusses und des Sees bei Kardoskút und rekonstruierte, unter welchen ökologischen Verhältnissen sie gelebt hatten. Diese Befunde bieten eine wichtige Ausgangsbasis für die Beurteilung der Faunenentwicklung der heutigen Theiss und ihrer toten Arme.

Die wichtigsten Erkenntnisse ANDOR HORVÁTHS sind seiner eigenen Darstellung nach wie folgt: obwohl "es im engeren Sinne genommene niveauanzeigende pleistozäne Molluskengattungen (im Bereich des Ungarischen Lösz-Beckens) nicht gibt, ist die Synthese dennoch möglich, weil die Molluskenfauna eine Periodizität aufweist". Diese Tatsache ist wichtig, da laut Feststellung von D. GEYER (1909) und ROTARIDES eine Synthese aufgrund der Pleistozän-Mollusken unmöglich ist. Bis zu den fünfziger Jahren hat man sich mit Forschungen in dieser Richtung weder in Europa noch in Ungarn beschäftigt. Die fossilen Gattungen wurden mit der heute unbrauchbaren Bezeichnung "pleistozän" versehen.

"Die Naturverhältnisse der einzelnen Perioden lassen sich anhand der Schnecken-Assoziationen rekonstruieren. Die Faunenperiodizität entspricht den periodischen Klimaveränderungen des Pleistozäns. Die Perioden sind identifizierbar mit den Perioden der MILANKOVITS-BACSÁK'schen astronomischen Berechnungen. Die Periodischen Wiederholungen des Klimawechsels bedeuten nur sich wiederholende Ähnlichkeiten, nicht aber sich wiederholende Identitäten. Die Synthese ist auch anhand einer einzigen Bodenprobenserie möglich, wenn die Proben in hinreichend kurzen Abständen entnommen wurden."

Die Ergebnisse seiner Untersuchungen zeigen, dass die aufgrund der Pleistozän-Mollusken erfolgende Synthese nicht nur in ungarischer Relation genutzt werden

kann. Seine Erfahrungen haben sich seit dem Erscheinen der Arbeit von V. LOZEK (1964) auf die europäischen Pleistozän-Forschungen fruchtbringend ausgewirkt.

Über seine Mitgliedschaft des Theiss-Forschungsausschusses hinaus war ANDOR HORVÁTH auch Verwalter des Theissforschungs-Zielkredits bzw. Ziel-Darlehens. Er war technischer Redakteur nicht nur der die Ergebnisse der Theissforschung veröffentlichenden Zeitschrift Tiscia, sondern auch der Acta Biologica Szegediensis der Universität. Er war Mitglied der Arbeitsgemeinschaft für die Erforschung der Natrongewässer und der Ungarischen Biologen-Vereinigung, sowie damals einziges ungarisches Mitglied der Unitas Malacologica Europaea (Europäische Malakologen-Gesellschaft).

Dem Andenken ANDOR HORVÁTH's haben seine Schüler und Kollegen drei Veranstaltungen gewidmet: 1983 hielt im Rahmen einer Vortragssitzung der Szegeder Biologischen Gesellschaft Prof. AMBRUS ÁMBRAHÁM eine Gedenkrede für seinen einstigen Schüler. Mit ihren Arbeiten erwiesen ihm seine Schüler: MIKLÓS SZÓNOKY, ANDOR RICHNOVSZKY, GYULA KOVÁCS und KÁROLY BÁBA die Ehre. Aus Anlass des 15. Todestages hielt 1987 sein einstiger Kommilitone DEZSŐ LUKÁCS im Rahmen einer Sitzung der Zoologischen Fachabteilung der Biologischen Gesellschaft in Budapest einen ehrenden Nachruf.

Anlässlich der 20. Wiederkehr seines Todestages gedachten seiner in einer Vortragssitzung der Biologischen Gesellschaft in Szeged seine Schüler Mihály Mucsi, ANDOR RICHNOVSZKY und KÁROLY BÁBA.

Sein Humanismus und sein Pflichtbewusstsein gelten seinen Schüler als Vorbild. Sein Andenken bewahren wir in Treue und tiefer Verehrung.

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COUPLED IMMOBILIZED ENZYME - IMMOBILIZED CELL SYSTEM FOR CONTINUOUS PRODUCTION OF ETHANOL

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Abstract

For the continuous production of ethanol from thinned starch, a column reactor filled with covalently immobilized glucoamylase was coupled with a vertical reactor segmented with perforated plates supporting Saccharomyces cerevisiae cells entrapped in calcium alginate. The operation of the system was characterized by a fermentation efficiency of 84.5 ± 4.1%, and an ethanol concentration of 38.8 ± 0.3 g/l,

Key words: glucoamylase immobilized, yeast immobilized, ethanol production, bioreactor

Introduction

Starch is one of the most important raw materials for industrial ethanol production.

Two enzymes are generally used for the production of glucose from starch. α - Amylase is employed in soluble form since the molecular weights of its substrates, amylose and amylopectin, are too high for satisfactory hydrolysis with immobilized enzymes (HARTMEIER, 1986). In contrast, glucoamylase can be applied in immobilized form for the continuous saccharification of starch previously thinned by α-amylase. The continuous production of ethanol is performed by immobilized microbial cells. Different vertical packed-bed and fluidized-bed reactors are preferentially used (GODIA et al., 1987). Successful pilot plant and industrial operations are known (NAGASHIMA et al., 1983; 1987; NAJIMA et al ., 1987).

The present paper reports a coupled immobilized enzyme - immobilized cell system for the continuous production of ethanol from thinned starch as substrate.

Materials and methods

Chemicals. Glucoamylase was isolated from Aspergillus niger with a specific activity of 900-1500 units g^{-1} protein. Akrilex C-100, a polyacrylamide bead polymer containing carboxylic functional groups (6.4 meq g^{-1} dry wt) was a commercial product of Reanal. Its molecular exclusion limit was 100,000 daltons. 1-Cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-4-toluene sulfonate was purchased from Serva Feinbiochemica GmbH (Heidelberg, FRG). Soluble starch was a preparation of E. Merck AG (Darmstadt, FRG).

Corn starch was a gift from the Research Institute of the Alcohol Industry. All other chemicals were reagent grade commercial preparations (Reanal).

Microorganism and culture medium. Commercial baker's yeast was used. The cells were grown in a water bath shaker at 30 °C in a culture medium containing 100 g l^{-1} sucrose and diffrent nutrients, as described by WADA et al. (1979). The pH was adjusted to 4.0. Cells were harvested by centrifugation at 2500 x g for 10 min.

Immobilizations. Glucoamylase was covelently immobilized on a polyacrylamide support (Akrilex C-100) containing carboxylic groups activated by 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-4-toluene sulfonate as described earlier (SZAJÁNI et al., 1985). The activity was 10.2 units g^{-1}. One unit is defined as the amount of enzyme required for the liberation of 1 g of D-glucose from soluble starch per hour at pH 3.8 and 60 °C. For immobilization, baker's yeast cells were suspended in a sterile sodium alginate solution (Protanal SF 120, protan and Fragertun A.S., Drammen, Norway). The final cell density was 1x 106 cells ml^{-1}. Beads (∅ 4 mm) were formed by dripping the suspension through a syringe into sterile 1% calcium chloride solution. The cells were grown in a water bath shaker at 30 °C for 24 h.

Thinning of starch. Technical grade corn starch (700 g) was suspended in 2000 ml water, and 4 ml Optitherm LT α-amylase (Miles Laboratories Ltd.) was added. The suspension was incubated at 60 °C for 10 min. The temperature was then raised to 80-90 °C, a further 4 ml α-amylase was added and the incubation was continued for 20 min. This treatment was repeated twice. The suspension was next boiled to stop the action of α-amylase and was filtered. The pH of the filtrate was adjusted to 4.0 - 4.2 with 1 M hydrochloric acid.

Analytical methods. D-Glucose was measured iodometrically (ERDEY, 1956) or with glucose oxidase. Ethanol was determined by gas chromatography, with a Chrom 4 gas chromatograph (Laboratomi Pristroje, Prague, Czech Republic) equipped with a flame ionization detector and a Porapak Q (80-100 mesh) column (250 cm long and 3 mm i.d.). Nitrogen was used as carrier gas and methanol as internal standard.

Results and discussion

For the continuous production of ethanol from thinned starch as substrate, two bioreactors were coupled together. The first was a column reactor (4 x 1.5 cm) filled with glucoamylase (125 mg dry) covalently immobilized on a polyacrylamide support activated by water-soluble carbodiimide (SZAJÁNI et al., 1985). The second reactor was a vertical one segmented with perforated plates supporting Saccharomyces cerevisiae cells entrapped in calcium alginate (BUZÁS et al., 1990). The reactor volume and the length/diameter ratio were 143 ml and 4.2, respectively. The total gel volume of 73 ml with a cell density of 1 x 108 cells ml^{-1} gel, was equally divided onto 3 perforated trays.

Thinned corn starch (glucose content 102 g l^{-1}, pH 4.0) was passed through the first, immobilized enzyme reactor at a flow rate of 2.8 ml h^{-1}. The column was maintained at 60 °C. In a reservoir, the effluent was cooled and diluted to about 9% glucose content, and calcium chloride solution was added to it to give a final concentration of 1%. The immobilized cell reactor was fed with this medium at a flow

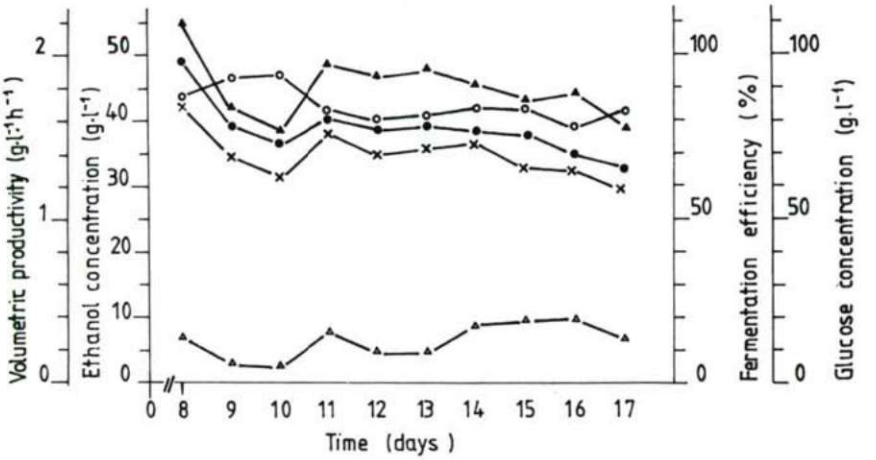

Fig. 1. Progress curves of the fermentation of thinned corn starch. Fermentation efficiency, O ; ethanol concentration, • ; volumetric productivity, x ; glucose concentration in the fermentation medium, Δ; and in the effluent, ▲.

rate of 5 ml h^{-1} at 30 °C. The two reactors were operated separately for 7 days to reach a steady state, after which they were coupled together and operated continuously. The progress curves of the fermentation are presented in Fig. 1.

The average values characterizing the process were found to be: fermentation efficiency, 84.5 ± 4.1%, ethanol concentration in the mash, 38.8 ± 0.3 g l^{-1} and volumetric productivity, 1.40 ± 0.12 g l^{-1} h^{-1}.

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EFFECTS OF ANTICHOLINESTERASES ON MUSCARINIC RECEPTOR BINDING PROPERTIES IN THE RAT BRAIN

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Abstract

Changes in the muscarinic acetylcholine receptor binding properties in response to *in vitro* administered cholinesterase (ChE) inhibitors were studied in rat brain homogenates. Several anticholinesterases (ethopropazine, 1,5-bis(4-allyldimethylammoniumphenyl)pentan-3-one dibromide, eserine, iso-OMPA and DFP) were tested to inhibit specific [^{3}H](-)quinuclidinyl benzilate ([^{3}H](-)QNB) binding to rat brain membrane preparations. Under the conditions applied, the relative affinities of these compounds were found to decrease in the following sequence: ethopropazine, 1,5-bis(4-allyldimethylammoniumphenyl)pentan-3-one dibromide, eserine, iso-OMPA. DFP did not affect the specific binding of [^{3}H](-)QNB *in vitro*. The K_i values for the individual drugs were: ethopropazine, 10^{-7} M; 1,5-bis(4-allyldimethylammoniumphenyl)pentan-3-one dibromide, 3.1×10^{-6} M; eserine, 2.1×10^{-4} M; iso-OMPA, 10^{-3} M. The results suggest that certain ChE inhibitors might be able to compete with specific [^{3}H](-)QNB binding at the muscarinic receptor.

Key words: anticholinesterase, muscarinic acetylcholine receptor, CNS, rat

Introduction

Several studies have demonstrated that the number and pharmacological properties of the muscarinic acetylcholine receptors (mAChR) in nervous tissues change under various pathological conditions, such as disease (WASTEK and YAMAMURA, 1978; RUBERG et al., 1982), chronic administration of drugs (GAZIT et al., 1979; NOMURA et al., 1979; EHLERT et al., 1980a, b), or certain *in vitro* circumstances (AGUILAR et al., 1980). For example, the chronic administration of cholinesterase (ChE) inhibitors to animals led to behavioral tolerance (OVERSTREET et al., 1974). It has been suggested that this phenomenon may result from a decreased sensitivity of the mAChR in response to the increased levels of acetylcholine (ACh; RUSSEL et al., 1980). In other experiments (EHLERT and KOKKA, 1978; GAZIT et al., 1979), the chronic administration of organophosphates led to a marked reduction in the density of mAChR in several regions of the rat brain, as revealed by the binding profile of [^{3}H](-)quinuclidinyl benzilate ([^{3}H](-)QNB). However, there relatively few

data are available concerning about the *in vitro* action of anticholinesterases on mAChR in the mammalian nervous system.

The main goal of the present study was therefore to investigate the *in vitro* effects of some of the most commonly used ChE inhibitors on the mAChR binding in the rat brain.

Materials and Methods

Male rats (CFY strain) weighing 180-200 g were used. The brains were quickly removed and homogenized (10% w/w) in ice-cold 0.32 M sucrose containing 0.1 mM EDTA in a glass homogenizer with a motor-driven Teflon pestle (6,000 rpm). The method used to study the binding of [^{3}H](-)QNB was a modification (GULYA and KÁSA, 1984) of that of YAMAMURA and SNYDER (1974). Briefly, the binding assay was performed in 50 mM sodium phosphate buffer (pH 7.4 at 25 °C) containing 0.5 nM [^{3}H](-)QNB (1.18 Tbq mmol^{-1}; Radiochemical Centre, Amersham, UK) in the presence or absence of various concentrations (10^{-11} to 10^{-3} M) of anticholinesterases. A second set was also prepared additionally containing 1 μM atropine, to determine the specific binding. The binding reaction was initiated by the addition of 50 μl of 0.1 % homogenate, and the incubation was allowed to proceed for 120 min at 25 °C. The incubation was terminated by rapid filtration of the mixture through a Whatman GF/C glass fiber filter. Each filter was washed with 5 ml of 50 mM sodium phosphate buffer (pH 7.4 at 4 °C) and then air-dried in scintillation vials. Ten ml of scintillation fluid (1000 ml of toluene, 150 mg of POPOP and 4 g of PPO) was added to each vial. The radioactivity was determined with an LKB 1215 Rackbeta II scintillation counter with 44% efficiency. Corrections for quenching were composed via a quench curve prepared by means of the external standard channel ratio method. The specific binding of [^{3}H](-)QNB was defined as the difference between the total and the nonspecific binding of the radioligand in the presence of 1 μM atropine. IC_{50} and n_H values were determined by indirect Hill (logit-log) plots of the inhibition of the specific [^{3}H](-)QNB binding by ChE inhibitors (GraFit 3.0, Erithacus Software, U. K.), and converted to K_i values via the equation $K_i=IC_{50}/(1+c/K_D)$, where c is the concentration of the radiolabeled ligand. Protein concentrations were determined by the method of LOWRY et al. (1951), using bovine serum albumin as standard.

The following ChE inhibitors were used: eserine sulfate, 10-[2-(dimethylamino)propyl]phenothiazine (ethopropazine hydrochloride), 1,5-bis(4-allyldimethylammoniumphenyl)pentan-3-one dibromide, tetraisopropyl-pyrophosphoramide (iso-OMPA), all from Sigma, St. Louis, USA, and diisopropyl-fluorophosphate (DFP; Fluka AG, Buchs, Switzerland). All drugs were freshly dissolved in 50 mM sodium phosphate buffer (pH 7.4 at 25 °C).

Results

The anticholinesterase agents were tested for their ability to displace specific [^{3}H](-)QNB binding to rat brain homogenate. The most potent inhibitor of specific [^{3}H](-)QNB binding among the anticholinesterases tested was ethopropazine (Table 1). Ethopropazine inhibited specific [^{3}H](-)QNB binding with a K_i value of about 10^{-7} M, while 1,5-bis(4-allyldimethylammoniumphenyl)pentan-3-one dibromide, eserine and iso-OMPA had K_i values of 3.1×10^{-6}, 2.1×10^{-4} and 10^{-3} M, respectively. DFP at concentrations ranging from 10^{-11} to 10^{-3} M failed to affect mAChR binding.

Table I: Inhibition of [^{3}H](-)QNB binding by anticholinesterases. The concentrations required to inhibit [^{3}H](-)QNB binding to the receptors by 50% (IC_{50}) were determined from log probit plots and converted to K_i values via the equation $K_i=IC_{50}/(1+c/K_D)$, where c is the concentration of [^{3}H](-)QNB), and K_d is its dissociation constant.

Drugs	K_i (M)	n_H
atropine	10^{-10}	0.97
ethopropazine	10^{-7}	0.85
1,5-bis(4-allyldimethylammoniumphenyl) pentan-3-one dibromide	$3.1x10^{-6}$	0.70
eserine	$2.1x10^{-4}$	0.80
iso-OMPA	10^{-3}	0.84

Discussion

We have shown that, beside their known inhibitory effects on acetylcholinesterase (AChE, EC 3.1.1.7), anticholinesterases can inhibit specific [^{3}H](-)QNB binding to mAChR as well. Although several reports have previously investigated the effects of ChE inhibitors, relatively few of them demonstrate the *in vitro* effects on the binding characteristics of the receptor. GAZIT et al. (1979) showed that chronic administration of Tetram reduces the number of mAChR in several areas of the rat brain, while UCHIDA et al. (1979) reported a decreased [^{3}H](-)QNB binding in the ileum of the rat after DFP treatment. DAWSON and JARROTT (1981), however, could not demonstrate changes in the pharmacological properties of the mAChR in the brain and ileum as a result of administration of this drug. SIVAM et al. (1983) described that *in vivo* chronic administration of DFP reduces the number of mAChR sites without affecting their affinity, but *in vitro* treatment fails to affect the mAChR binding. In contrast, EHLERT and KOKKA (1978) and EHLERT et al. (1980a) reported that chronic DFP treatment decreased the [^{3}H](-)QNB binding in the striatum; however, they emphasized that this decrease was not due to a direct effect of DFP on the mAChR, since no inhibition of binding was produced by high concentrations of DFP added *in vitro*.

In our experiments, DFP did not influence the specific binding of [^{3}H](-)QNB *in vitro*, but another organophosphorus compound examined, iso-OMPA, did have a slight effect. The ChE inhibitors which compete with the binding of [^{3}H](-)QNB *in vitro* were ethopropazine, 1,5-bis(4-allyldimethylammoniumphenyl)pentan-3-one dibromide and eserine. The most potent of these was ethopropazine, which is in good agreement with earlier reports of its weak atropine-like effect (SILVER, 1974).

ChE inhibitors are widely used in physiological and pharmacological experiments in order to detect the effects of ACh electrophysiologically. SZERB and SOMOGYI (1973) were able to show that, when the AChE activity of rat cortical slices was inhibited with eserine, the release of ACh evoked by electrical stimulation was slightly depressed. As our results reveal, this may be due to the direct competitive effect of the anticholinesterase drug on the pre- and postsynaptic mAChR of cortical

cholinergic neurons and draws attention to the limits of its applicability in physiological experiments.

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POPULATION GENETICS: FACTORS OF HUMAN EVOLUTION

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Introduction

We tend to describe individuals, their morphology and their pathology. In fact, we are not living as individuals, but as social beings, our unity being population. Fossil remains were not always "fossils"; they once belonged to living populations, and prehistoric man did not know they were prehistoric.

The anthropologist is interested in describing the variability of human populations and their origins. However, the definition of a human population is difficult.

The human species is a group of individuals, where all marriages are potentially fertile. However, these unions are limited by numerous barriers of geographical, socioeconomic, ethnical and psychological origin. These barriers would limit isolates if they functioned perfectly: in fact, they are never totally closed and the endogamy is never perfect. Therefore populations have to be defined by their barriers (JACQUARD, 1974, 1977; SUSANNE 1986).

Dimensions of populations

These presence of different barriers results in a limitation of the choice of a partner and thus of the dimension of a population.

We can estimate that throghout evolution the number of individuals in each population was very limited (Table 1). In fact, in our industrial societies, marriage groups (cercles de marriages) are also reduced.

To understand evolution better, the number of individuals is not sufficient information; it is important to know the repartition of sex and age too. If, in a large population, one can estimate that the distribution of sexes is equilibrated, at least at the age of procreation, in limited population the random distribution could in contrast be totally disturbed. Moreover, in wartime, a serious loss of young men can induce an abnormal sex ratio.

Table 1. Estimation of size and of density of populations

Size	
Chimpanzee	20-40
Early Homo	20-50
Veolithicum (village)	50-1000
Amazonia	20-80
Pygmies (camp)	10-100
Australian aborigenes	20-50
Maya (village)	500
New Guinea (village)	100-300
Belgium (marriage group)	300-1000
Density per km^2	
Chimpanzee	0.07-0.09
Omo (Australopithecus)	0.006-0.016
Hunter-gatherer	1
Early agriculture	10
Traditional agriculture	40
Belgium	1000
Hunter-gatherer	
Pygmies	0.2
Eskimo (Caribou)	0.04
(Greenland)	0.06
(Aleut)	0.6
Australian aborigenes	0.03
Primitive agriculture	
New Guinea	4
Maya	20

Therefore, it is necessary to define the effective number of individuals. The probability that two genes of two different individuals come from the same male is 1/4 Nm (with Nm the number of males), and from the same female is 1/4 Nf (with Nf the number of females). The two genes thus come from the same individual with a probability of

$$\frac{1}{4Nm} + \frac{1}{4Nf} = \frac{1}{4Ne}$$

where Ne is the effective dimension of the population.

$$Ne = \frac{4NmNf}{Nm + Nf}$$

This value will in fact depend essentially on the lowest values of Nm or of Nf, and can differ profoundly from the total number of individuals (Table 2).

Table 2. Effective size in function of the number of males (Nm) or females (Nf) for a total N=100.

N	Nm	Nf	Ne
100	1	99	3.96
100	5	95	19
100	10	90	36
100	30	70	84
100	50	50	100
100	70	30	84
100	90	10	36
100	5	95	19
100	1	99	3.96

Law of HARDY and WEINBERG

The theory of population genetics is based on the classical low of HARDY and WEINBERG, defining the situation where the frequency of genes would remain constant (Table 3).

Table 3. Law of Hardy and Weinberg

1) Let us suppose 2 alleles A_1 and A_2
with frequency p_1 and p_2 with $p_1+p_2=1$
The genotypes A_1A_1 A_1A_2 A_2A_2
will have as frequency p_1^2 $2p_1p_2$ p_2^2

In this new generation, the frequency of A_1 is

$$p_1' = \frac{2p_1^2 + 2p_1p_2}{2} = p_1(p_1 + p_2) = p_1$$

and of A_2

$$p_2' = \frac{2p_2^2 + 2p_1p_2}{2} = p_2(p_1 + p_2) = p_2$$

The frequencies of genes are constant.

2) Let us suppose panmictic marriages

	Frequency	Frequency of children		
		A_1A_1	A_1A_2	A_2A_2
$A_1A_1xA_1A_1$	p_1^4	p_1^4		
$A_1A_1xA_1A_2$	$4p_1^3p_2$	$2p_1^3p_2$	$2p_1^3p_2$	
$A_1A_1xA_2A_2$	$2p_1^2p_2^2$		$2p_1^2p_2^2$	
$A_1A_2xA_1A_2$	$4p_1^2p_2^2$	$p_1^2p_2^2$	$2p_1^2p_2^2$	$p_1^2p_2^2$
$A_1A_2xA_2A_2$	$4p_1p_2^3$		$2p_1p_2^3$	$2p_1p_2^3$
$A_2A_2xA_2A_2$	p_2^4			p_2^4
		p_1^2	$2p_1p_2$	p_2^2

The frequencies of genotypes are constant.

The absence of changes in the frequency of genes corresponds to the absence of evolution occurring when no migration, selection or mutation occur, when the

population is large and marriages occur at random (panmixy). This situation is therefore theoretical and the low allows us to study what influence the absence of respect of the conditions could have in terms of evolution.

The study of evolution implies control of the different conditions of the low of HARDY and WEINBERG, but esentially natural selection, mutation and migrations. However, we would like to insist on the random factors linked to the presence of populations of limited dimension.

History of family or history of genes

A family always has a complex history, where each individual has two parents and where, after n generations, each individual can have 2^n ancestors. However, the history of a gene better describes the biological evolution and is easier to follow because of the fact that each gene has only one ancestor gene.

Figure 1 compares an example of genealogy with the history of genes, where we can observe the disappearance of some genes and the multiplication of others.

Genetic drift

As we have seen, an essential part of the history and prehistory of man occurred in populations of limited dimensions, generally around 50 individuals (Table 1). When a population becomes larger, migrants leave the initial group for new territories: this migration results in a random choice of a limited number of genes. The "child" population will have a genetic pool different from the pool of the "mother" population.

Table 4. Probability of absence of transmission

at individual level

a parent to a child	1/2
from a parent to n children	$(1/2)^n$

at population level
probability of absence of transmission

$$p = \sum_1^x \frac{\lambda x}{2^x}$$

with λx the proportion of the population having x children

Examples of 2 populations having average 2 children

A with l2=1

$$p = \frac{1}{2^2} = 0.25$$

B with l0=1/2l4=1/2

$$p = \frac{1}{2} \cdot \frac{1}{2^0} + \frac{1}{2} \cdot \frac{1}{2^4} = 0.53$$

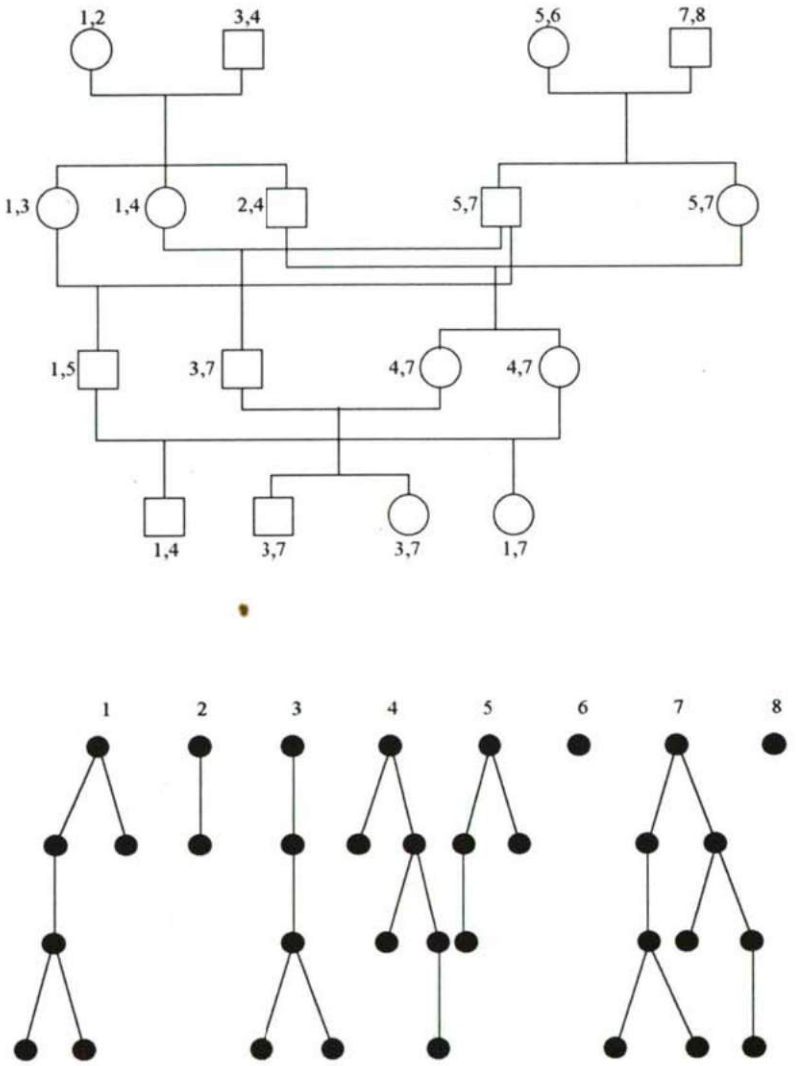

Fig. 1. Above: history of a family, each individual can have 2n ancestors.
Under: history of a gene (represented by numbers), one gene has only one ancestor gene.

A new generation is also always a result of a random choice of the gametes implicated in the fecundation and thus in the new zygotes.

Such a random choice in a very large population will not influence the frequency of genes. However, when the population is very limited, the random factors may result in large fluctuations of frequencies of genes. This situation is known as genetic drift; it corresponds to the random absence of transmission of some genes and to the random fixation of others.

At the limit, it becomes more important to follow the absence of transmission itself. The chance for a gene at a specific locus not to be transmitted to a child is 1/2, and to n children is $(1/2)^n$.

At the level of a population, the probability of absence of transmission is

$$p = \sum_{1}^{x} \frac{L_x}{2^x}$$

where L_x is the proportion of the population having x children. This probability depends on the number of children (x), but also on the variation in the number of children (Table 4).

Table 5. Variance of the frequency p_i of a gene after one generation of reproduction in a population of dimension N.

$$V(p_i) = \frac{p_i(1-p_i)}{2N}$$

Example 1: p_i=1/2 with 95%		Example 2: p_i=1/4 with 95%	
N=5	0.3 à 0.7	N=5	0 à 0.52
N=10	0.32 à 0.68	N=10	0.0 à 6 0.44
N=50	0.42 à 0.58	N=50	0.12 à 0.38
N=100	0.44 à 0.56	N=100	0.17 à 0.33
N=500	0.48 à 0.52	N=500	0.19 à 0.31

Random factors can also influence the frequency of a gene in a population, and this is inversely proportional to the dimension of the population. In a population of dimension N where a gene a_i is present in n_i copies, its frequency is equal to $p_i=n_i/N$.

In the new generation, the probability of frequency of the gene is expected to be equal to the previous frequency, but its variance is a function not only of this frequency, but also of the dimensions of the population (Table 5).:

$$E(p_i)=p_i$$

$$V(p_i) = \frac{p_i(1-p_i)}{2N}$$

If the random fluctuation can be estimated from generation, following the rules of binomial distribution, it becomes more difficult to estimate the evolution of gene

frequencies after many generations. Computer simulations have been used to demonstrate that the final result of genetic drift is fixation of one allele and loss of the other one.

Founder effect

A specific example of genetic drift is the founder effect: our history contains many cases of migrations of limited number of individuals, founding a new population and thus participating in an effect of genetic drift.

The Jicaque population (CHAPMAN et al., 1971) gives a tipical example of the founder effect, but also of a history of genes, where as a function of variation in fecundity and mortality the structure of a population modifies itself.

The Jicaque were founded a century ago by only 7 persons, who isolated themselves and their descendents on a voluntary basis; their genealogy has been reconstructed. Table 6 indicates the number of persons by generation and the level of consanguinity. This consanguinity naturally depends on the low dimension of the population; it is diminishing in the 5th generation under the influence of some migrants. Table 7 is in fact richer in information. It reconstructs the probability of origin of the genes issuing from the 7 founders, and shows that from the 2nd generation the weights of the different founders can fluctuate considerably (with a factor 2.5 in the 2nd generation, and a factor 5 in the 5th generation).

Table 6. Consanguinity and effective size of the Jicaques population.

	Consanguinity	Nm	Nf	Ne	N
1	0	4	3	6.94	7
2	0	19	15	33.5	34
3	0.068	96	80	174.5	176
4	0.092				247
5	0.065+				101

+ This diminution is due to the input of migrants

Table 7. Probability of genes originating from the founders of the Jicaques population.

		Founders								Migrants	
Generation	Nb	Léon	Franc.	Caciana	Juan	Polinaria	Pedro	Petrona	Total	Indians	Hybrids
1	7	143	143	143	143	143	143	143	1000		
2	34	88	74	74	191	176	199	199	1000		
3	176	66	78	78	167	151	208	208	957	43	
4	247	54	68	68	162	146	195	195	888	81	31
5	101	24	31	31	132	126	129	129	602	186	212

The Touaregs Kell Kummer (CHAVENTRÉ, 1972) present a relatively similar example of the founder effect and of genetic drift. At the end of the 17th century, a dominant group of Touaregs (the Kell Tademaket) lived in the South Sahara. A group seceded under the leadership of a man called KARI DENNA. This group (the Kell Kummer) slowly imposed its domination and migrated to the South of Mali. Under the

French occupation at the end of the 19th century, the Kel Kummer resisted and many were killed. In 1970, only 367 individuals (171 men and 196 women) were still living. The whole population is in fact based on 156 founders. Table 8 gives a summary of the origins of the genes of the different founders. The asymmetry is evident, since KARI DENNA contributed to 11-20% of the gene pool, while only 15 founders contributed to 70% of the gene pool and 25 founders to 80%.

Table 8. Touaregs Kel Kummer: Origin of genes (in ‰)

Generation	1 à 5	6-7	8-9	10-11	12-13	14 à 16
Size of the population	126	376	601	653	268	245
Founders						
1093 & 1096	198)	142)	158)	146)	155)	186)
1 & 2	199)571	110)350	116)370	105)357	105)361	116)426
1331 & 1332	174)	98)	106)	106)	101)	124)
1919	30	66	76	64	71	107
1959	47	55	70	60	63	71
2060	16	60	48	33	37	33
2067	16	21	49	45	43	46
1968	29	35	39	34	40	53
2062	0	44	25	24	25	27
1628 & 1629	38	20	19	15	19	24
2063	0	23	23	15	22	20
Whole of the 15 mains	747	674	729	647	681	807
Whole of the 10 following	103	112	102	111	101	78
Whole of the 131 others	150	214	169	242	218	115

1-2 = Kaari Denna and his wife
1331-32 = parents of the wife of Kaari Denna

In fact many other examples of the founder effect are known. The Hutterites, for instance, are a group of anabaptists who migrated from Europe to the United States in 1872. Three colonies were formed, with a large genetic variability from the origin on (STEINBERG et al., 1967). The religious sect of Old Order Amish migrated to Pennsylvania between 1720 and 1770; it created numerous isolated colonies where a recessive pathology is frequently observed (MCKUSICK et al., 1964). The Dunker are another sect who migrated during the 18th century; 55 groups of less than 100 persons were founded, and an important genetic drift explains the genetic variability observed between these groups (GLASS, 1954).

The high frequency of retinitis pigmentosa in the population of Tristan da Cunha (ROBERTS, 1968), of porphyria in the Africaners, and of chorea of Huntington in Tasmanians also result from a founding effect. Porphyria for instance, was introduced into the Africaner population by a migrant couple; GERRIT JANSZ, a Dutch farmer, arrived in Kaapstad in 1688 and married an orphan named ARIAANTJE JACOBS. The chorea of Huntington of the Tasmanians originates from a Huguenot woman who left England in 1848.

The distribution of some blood groups also finds its origin in genetic drift: this is the case with the group O more frequent in Amerindians and rhesus r' and r being absent in the the same Amerindians.

A study of the non-coding DNA situated near the B globin gene shows four variants that are very frequent in human populations: three are present with the same frequency all over the world, while the fourth is only present in Africans. It is possible to estimate that a founder effect was generated by the migration of human populations of *Homo erectus* from Africa. It is even possible to estimate the number of migrants: they were probably about 50 during about 70 years or about 500 during 200 years. These numbers look astonishly low, but are indeed probable (FLINT et al., 1992; ROUHANI et al., 1992).

Migration

If the founder effect corresponds to a history of fissions of populations, the migration corresponds in contrast to a history of fusion. The isolates are never totally closed; exchanges between populations still occur. These migration can temper the genetic effect of gene drift; they have a homogenizing effect.

Conclusion

A population always tends to a balanced situation under the influence of selection, migration and genic drift. However, different populations respond to different balances under the influence of varied effects of selection, migration and genic drift: the random factors are no longer factors of uniformization, but of diversification.

The isolation of a population is never total; some genetic contribution of migrants will always be observed, even if the level of migration is continuously changing. This has the result that no region is sufficiently stable to result in a balanced situation.

The (pre)history of human populations is a history of relatevly isolated populations, of limited dimensions and thus of genetic drift. It is also a history where the culture and socioecological factors result in fissions and fusions: fissions are factors of the breaking-down of endogamy and thus of diversification, while fusions are factors of homogenization.

Our unity is and always has been the population. These populations have never been fossils.

Addendum

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BODY COMPOSITION: HISTORY, METHODS AND APPLICATIONS

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History

Before I begin to talk about the history of the body composition, and researches which are, from my point of view, an important line of investigation within the field of anthropology, I think it is necessary to define the concept of body composition.

For me, it would be "The evaluation of the different components of the body, but considered as a whole. The analysis is done with consideration to the chemical, physiological and morphological changes that act on the body."

(If I have not mentioned the numerous methods of measuring body composition yet, it is because I will talk about them later.)

Historically, since the closing years of the 19th century, studies have been performed with the aim of learning the biochemical changes occurring in the body with age. These studies were carried out on foetuses and newborn babies. During the first third of the present century, metabolic balance techniques were used to quantify the composition of the mass put on during a gain in weight. From that time, studies by the Czech anthropologist MATIEGKA (1921) are particularly noteworthy. This author was the first to make an estimation of the different main components of the body weight on the basis of anthropometric data. - And this, in a period when anthropology was mainly osteologic and descriptive, was simply revolutionary.

During the 1950-s the studies by SKERLJ and BROZEK were also important for they reinvestigated body composition. BROZEK (1952) pointed out how the analysis of body composition greatly increases the field of application of physical anthropology, giving it a biomedical meaning, helping as to know the intraindividual and interindividual alterations in weight related to age, environment, activity, sex, etc.

In the 1960-s, the methods of determining body composition were set, as were a great number of applications of these measurements: a) Sports Centers, b) nutrition and bromatology, c) medical sciences: paediatrics, gerontology and forensic medicine.

Which techniques were thought up to measure and study the mass between the skin and the bones?

Body weight was almost the only measurement taken into consideration by paediatricians, internal physicians and others to characterize a live individual, though, as BROZEK pointed out, weight can be mistaken even when related to a length (height)

of a certain individual (nevertheless, weight for height and the ratio height/weight are still common indices in clinics).

For instance, in an adult person, the body weight for height can increase for different reasons, such as:

1. Changes in the "body scaffolding", i.e. an increase or decrease in the bony mass.

2. Increased muscular tissue (after a period of exercise).

3. Increased fat tissue (caused by a metabolic disease, a sedentary life or bad nutritional habits).

A decrease in body weight in an adult person can be a result of many different losses, with very different implications:

1. Loss of tissular water (as occurs, naturally, during the ontogenetic processes from birth to old age) or due to pathologic causes.

2. A diminution in the amount of muscular tissue (e.g. during periods of inactivity or when a physical effort ceases).

3. A diet restriction, which affects the fat tissue or, in certain cases, other tissues (undernutrition).

Of course, all these factors have very different biological meanings.

The inclusion of other anthropometric measurements, such as the trunk circumference did not help much to clarify the picture. There was something else to know, something that defines the shape of every individual: the fat tissue.

WIDDOWSON (1968) analysed the subcutaneous fat precipitate from dead foetuses aged 28 to 40 weeks and observed that the amount of fat increased with time (age).

BEHNKE (1942) studied great depth divers and worked out the basis of the equations later applied for the analysis of the human weight divided into its two fractions: fat weight and lean weight, through total body weight, body volume and total body water.

Related to this field, I must also mention the work of LJUNGGREN (1957) on nutritional anthropology, where the variation in body composition was analysed during the growing process and through the metabolic balance.

All the above-mentioned findings lead us to think that there is great agreement in considering the body weight divided into two main fractions.

Fat body weight

Fat, as a fraction of the body weight, but also as a component of the fat tissue, and a part of the nervous fibres, viscera, etc., has fundamental missions not only as an isolator, but also as an energy storage that gives to the organism a certain independence from the environment.

Its density is practically constant: 0.9×10^3 kg/m^3. The fat mass greatly varies not only interpopulationally and interindividually, but also in a given person throughout his life and/or depending on situations of organic stress. Fat tissue also shows a clear sexual dimorphism related to the hormonal changes in adolescence.

Lean body weight

This is the body weight minus the fat weight. Hence, the lean body weight includes: osseus tissue, muscles, mineral fractions, water, etc. It is evident when considering fat-free tissues (which particularize only the mass exempt of fat) that the lean tissues contain a small amount of fat (2%: essential lipids as lecithin and phospholipids).

In practice, the two concepts are often equal. The lean tissue density is approximately 1.10×10^3 kg/m^3, containing 68 milliequivalents of potassium for every kilogram of lean tissue in males (10% less in females), and 720 g of water. Its composition is 20% proteins, 7% minerals and 1% carbohydrates, the rest being water.

Evidently, the lean mass also shows a great variation, as observed in the fat mass. The factors that must be considered for both (lean and fat) tissues are as follows:

age, sex, race/ethnics, physical activity, exercise, nutrition, disease, social context.

Age

By the seventh month of intrauterine life, the foetusis rapidly increasing in weight. This occurs basically by an augmentation in adiposity, rather than in lean tissue. To a certain extent, the tissues dehydrate, their water content decreasing from 94% to 82% in the eight month of pregnancy.

The percentage body fat at birth is approximately 13%. This increases later, during the neonatal period.

Between 9 months and the first year of age, a negative rate of growth of the fat tissues is usually observed, the lean body mass then increasing (the child starts to become more slender). The loss of water continues during the first year of life, and at the end of this year the water content is about 74%. After another year (children aged 2 years), the water content has decreased to 60%.

During the growing process, there is an alternation between periods with increasing fat storage and periods with increasing lean tissue. For instance, between 3 and 4 years of age, children tend to be more ectomorphous; at 5-6 years, mesoendomorphous, etc., though it is certainly variable and dependent on the growing rhythm and speed for every individual. Thus, the top growing speed for lean tissues occurs at some undefined age, during the period known as adolescence, when important changes in fat tissue and total body weight also happen, in both males and females. There is a loss of water, more evident in females than in males.

In adult people, variations in body composition may be distinct from the events of growing. Thus, the augmentation of fat stores is mainly due to environmental factors, exceptions being pregnancy and breast-feeding periods in women.

The fat tissue remains almost constant through the whole adult life. In the last involutive period, two interesting phenomena develop:

1. Re-distribution of the fat tissue at about the age of 40-60 years. The fat tissue is still centralized, but this tendency increases the internal fat of the abdominal wall.

2. The lean tissue starts its deterioration after the fourth decade of life, leading to

a progressive decrease in height and some other partial lengths and a net deterioration of the muscular tissues, which is especially clear in old age.

Sex

This sort of genetically determined variation is not significant during the intra-uterine period and at birth (though there is a slightly higher lean content in males than in females). Howewer, such dimorphism increases during the growing process, the difference being 1% at the end of the first year of life, and 6% at pre-puberty (10 years). These differences accelerate considerably at adolescence, generating a predominance in the total body fat (internal and subcutaneous) in girls.

Although fat tends to centralization in both sexes, in women fat accumulation is more peripheral and goes together with a lower lean mass development in both bone and muscle. In males, lean mass development is prevalent, and though males have a lower fat tissue proportion than females, it shows a central distribution, generating different fat-storage patterns for men (apple-shaped) and women (pear-shaped), the etiologic consequences of which on morbidity can be regarded as risk factors for cardiovascular diseases.

These adult characteristics remain invariable during maturity and until the last stages of old age, when a clear diminution in fat, bone and muscular tissues is observed in both sexes.

Race/ethnics

It is difficult to talk about any racial differences in our species because of the great heterosis, and we cannot find "pure races". We are all somewhat half-breeds, "mestizos", because of migrations and other changes. Nevertheless, individuals with negroid ancestors (and so, clearly negroid individuals) show a higher lean/fat development. In europoids, higher accumulations of fat/lean mass can be observed, defining thereby a different structural prevalence in these groups, with larger shoulders and hip widths in negroids than in caucasoids. I do not dare to talk about a physical pattern, because many anthropometric data are related to a different socio-economic context in the two groups, which is always associated with the type of nutrition, type of work and quality of life.

This makes us think of the importance of analysing not only the racial context of body composition, but also the ethnicity (e.g. gipsy populations in Spain).

Physical activity/exercise

It is interesting to study this point, mainly if it is considered to be a marker, first of lower fat stores which are metabolized during the physical stress, and then, because of the higher lean development being basically muscular, muscle hypertrophy.

Not all the types of activity produce the same individual pattern, or rather, not every individual has a good predisposition to practise a certain activity. For example, a swimmer's body composition is different from that of a long-distance runner, and both are different from the body composition of a sedentary office worker.

The physical activity of an individual must be considered when estimating diets and body composition.

Nutrition

"Tell me what you eat and I will tell you who you are" says an old Spanish proverb. There is evidently a great deal of truth in this assertion.

The mode of nourishing presupposes a supply of the "bricks" (of one kind or another) to build our body, though with some safeguards.

Overnutrition (eating more than needed for the organic maintenance and the physical activity) leads to overweight, which, if not rectified, becomes obesity, with important increases in relative and absolute proportions of fat tissue.

Undernutrition causes an energetic demand that is mitigated by using the stored fat (emagramiento). If undernutrition persists, then the lean fraction is affected and there is a severe organic loss.

Disease

It is known that certain diseases also cause changes in body composition. For example, in children and still growing individuals it occurs, in certain environments, because of dehydration processes caused by diarrhoea, loss of fat and lean tissues caused by vomiting, due to infections, parasites, etc.

In adults, diabetes or gout produce alterations in the body fractions or in their distribution, as in the case of cardiovascular diseases (in this case, it is thought that it is the fat distribution that involves the higher or lower risk).

Social context

We are all familiar with the First World pattern versus the Third and Fourth World ones, i.e. injustice reflected in different body frames.

Individuals who live in a propitious environment have a greater lean development together with a larger fat store (even excessive) as compared with those of individuals from the same environment but belonging to a lower social class.

It has been like this until very recent times, when the lower classes from developed environments display a larger fat development and a higher tendency to obesity. This pattern has also been observed in studies made on recent "Western impact" areas, such as Polynesia, or in rural communities that have emigrated to industrialized areas.

Determination of body composition

Different work methods have been developed to determine body composition. These can be resumed in two different methodologies, with different fields of application:

1. Direct method

This method is applied in corpse dissection and chemical analysis of the corpse.

Starting from dissection, body fractions are evaluated as percentages of the total body weight. For instance, from the dissection of two caucasoid individuals aged 35 and 45 years, respectively, the fractions of the total body weight obtained were:

skeleton	14.8%, 17.6%
skin	7.8%, 6.3%
fat tissue	13.6%, 11.4%
striated muscle	31.6%, 39.8%
rest of the body	32.2%, 24.9%

To integrate these indirect data with the live individual, it is usually necessary to characterize the body composition in chemical terms (e.g. proteins, 11 kg; fat, 9 kg; carbohydrates, 1 kg; water, 40 kg; minerals, 4 kg; for an individual weighing 65 kg, that is: 17.6% proteins, 13.8% fat, 1.5% carbohydrates, 61.6% water and 6.1% minerals).

2. Indirect methods

These are used to estimate body composition in live individuals. There are sundry methods, all of them involving narrower or wider margins of error.

The first methods applied were:

A) Anthroposcopic-photoscopic methods

The technique consists in direct surveying or pictures and then, reference pictures to quantify the physical frame.

The obvious disadvantage of the method is the observer's subjectivity, which makes the comparison of data analysed by different observers less reliable.

B) Photogrametry

Starting from this method, SHELDON (1950) developed a typologic system: the somatotype, which, though not a proper method to determine body composition, has contact points with it.

With use of the somatotype, fat tissue (endomorphism), muscles (mesomorphism) and linearity (ectomorphism) are evaluated. The method starts from the concept that an individual has a constant morphogenotype during his ontogeny, provided that the conditions of energetic expenditure and nutrition are kept invariable (in practice, this is really difficult, and almost impossible).

In 1955, BROZEK derived an equation to the predict body density and the total body fat through endomorphism.

C)Radiogrametry

This is based on the study of X-ray plates, in which it is possible to evaluate subcutaneous fat, bone and muscle in terms of linear dimensions (thickness) and in transversal areas, allowing an estimation of the volumes of tissues.

STUART and REED started these studies in 1940 (1951), REYNOLDS in 1948 and GARN in 1954 (1956).

Such estimations can be advantageous since:

- Examination of the fat tissue is possible in areas of the body inaccessible to

other kinds of determinations (subcutaneous skinfolds).

- The technique is not complicated and the procedure is feasible despite the ontogenetic differences in the compressibility of the fat tissue.

- X-ray plates of extremities allow on evaluation not only of the fat, but also of the muscle and bone volume and size.

Nevertheless, there are some disadvantages to this method: First, the method is actually considered to be "invasive", and so it is accepted only in very concrete cases. Second, this technique is very expensive: X-ray plates and the necessary installations are not cheap. And third, the distances must be perfectly calibrated in order not to distort the real readings.

There are data from the 1950's and 1960's that are useful for the study of fat tissue regionalization.

Starting from this method, formulae have been calculated to estimate body composition:

GARN model: Based on a linear equation, with data obtained from X-ray plates:

$$W = a + bs$$

where W is the weight, b is a constant and s is the amount of fat tissue.

If s=0, then a = lean body weight

and $W = L + A$ (adiposity).

Though the model does not fit reality, it is true that the two body fractions (fat and lean mass) are involved.

D)Anthropometry

This consists in the use of anthropometric techniques together with other indirect methods to determine body composition:

- Linear measurements to determine fat tissue development: subcutaneous skinfolds.

- Musculature and robusticity: diameters and circumferences.

Subcutaneus skinfolds

About half of the stored fat is subcutaneous. This allows an easy measurement due to its accessibility. Given that the skin thickness is constant, the amount of fat stored can be evaluated from the thickness measured with a special skinfold caliper.

When this method is used, it is important to differentiate well between muscle and fat, and this requires some practice by the anthropometrist.

The number of points (skinfolds) to be measured depends on the specific work to be done, but the I.B.P. (International Biological Program) usually demands inclusion of at least the tricipital and subscapular skinfolds (for extremities and thorax fat stores) and also recommends inclusion of the suprailiac skinfold.

The most frequently used calipers are the HOLTAIN and LAUGE instruments. They both have a pressure of 10 g/mm^2 and it adds another difficulty to the measurement that the fat tissue "moves" during the first measurement, so the following measurement of the same skinfold must be done after an interval. Further, in obese people, it is difficult to include the last stratum of subcutaneous fat, which is very often omitted.

Musculature

Muscles can be characterized in terms of widths or diameters, with calculation of the transversal surfaces of the segments starting from the circumferences and subcutaneous skinfolds.

BROZEK established that, if the extremity is a cylinder, then from the known circumference, the diameter can be calculated, though the fat portion (the subcutaneous skinfold measure) must be corrected for:

$$c = \pi \cdot d \qquad d = c/\pi$$

$$d = c/\pi - s$$

JELLIFFE (1969) derived the following equations:

CA = circumference of the arm
TS = tricipital skinfold
MCA = muscular circumference of the arm
MDA = muscular diameter of the arm
MSA = muscular surface of the arm
FSA = fat surface of the arm
TSA = total surface of the arm

$MCA = CA - (\pi \cdot TS)$ $MDA = CA/\pi - TS$

$MSA = (\pi/4) \cdot (MDA)^2$

$FSA = (TS \cdot CA/2) - (\pi(TS)^2/4)$

$TSA = MSA + FSA = (CB)^2/4\pi$

In certain studies, other less familiar formulae have been used, such as the energy/protein index, etc. These formulae have an important disadvantage: the muscle and bone values cannot be eliminated.

Body frame: weight/height

The deviation of an individual's body weight referred to the standard value for a given height is useful for a rough estimate of his body composition.

These relationships belong in classical anthropometry:

- BOUCHARD index = weight/height
- Quetelet index or body mass index (B.M.I.) = $weight/height^2$
- ROHRER index = weight (kg)/$height^3$ (m^3)

In terms of nutrition, these indices can give a proper diagnostic criterion. The handicap is not to be able to differentiate between weight due to fat, muscle or oedema.

In 1982, KATCH and FREEDON developed a mathematic model to define the body architecture: the body frame. In males and females, this correlates the height, biacromial and bitrochanteric diameters, classified in categories, with the body weight, percentage of fat and lean body weight.

Differences in weight for the categories were due to the lean mass in men, and to the fat mass in women.

Some inaccuracies have been observed in the Actuary Society Tables. In 1983, the elbow diameter was included.

Nowadays, it is thought that the wrist and ankle better meet the aim of eliminating the weight free of fat (HIMES and BOUCHARD, 1985).

Tissue mass estimation

Densimetry techniques

These techniques appeared early (1949) and a great number of prediction formulae have been developed from them. The method is based in Archimedes' theorem, which estimates body densities from the volume displaced inside a fluid (D = M/V), so it is necessary to weigh the individual in water and then correct for the weight of the residual air in the lungs, estimating that:

$$\text{Density} = \frac{W_{ta} \times 0.996}{W_{ta} - W_{tb} - (V_{alr} \times 0.996)}$$

W_{ta} = weight out of water
W_{tb} = weight under water
V_{alr} = volume of residual air
0.996 = water density at 37 °C.

Once density is calculated, the amount of fat as a fraction of the total weight can be obtained by means of a general equation. This allowed the development of formulae to find the percentage of fat or the total fat weight:

Examples:

- KEYS and BROZEK (1953):

$$\%Fat = (4.95/d - 3.813) \times 100$$

- SIRI (1956) produced the most widely used formula:

$$\%Fat = (4.95/d - 4.50) \times 100$$

- Minnesota (this equation estimates density from the skinfold values):

$$\text{Density} = 1.0084 - 0.00071X_1 - 0.00048X_2 - 0.00055X_3$$

X_1 = mid armpit skinfold
X_2 = thorax skinfold
X_3 = tricipital skinfold

Later, PARIZKOVA (1971) devised a reliable estimation from the measurement of ten subcutaneous skinfolds, which took into consideration differences due to age and sex. Such a great number of measurements made the sampling very difficult. The skinfolds measured were: face, neck, thorax-1, triceps, subscapular, thorax-2, abdomen, suprailiac, thigh and calf.

Children (9-12 years)

Girls: %Fat = 2.399X - 2.457

Boys: %Fat = 2.660X - 3.134

Adults (17-45 years)

Women: %Fat = 39.572X - 61.25

Men: %Fat = 22.32X - 29.00

where X = log Σ skinfolds.

One of the most commonly used methods of prediction is that of DURNING and RAHAMAN (1967), which needs four subcutaneous skinfolds (bicipital, tricipital, subscapular and suprailiac) to calculate density, and, as before, X = log Σ skinfolds.

This possibility was enlarged in 1974 by DURNING and WOMERSLEY to facilitate working with equations in a wide range of ages (16 to 72 years) and including a great variety of types: workers, sportsmen, dancers, students, etc.

A total of 180 equations for body density were obtained with different number of skinfolds combinations.

For example, for the four skinfolds mentioned above, the formulae and constant values calculated for both sexes were:

density = c - m x log Σ skinfolds

Males

	Age (In years)					
	17-29	20-29	30-39	40-49	50+	17-72
c	1.1620	1.1631	1.1422	1.1620	1.1715	1.1765
m	0.0630	0.0632	0.0544	0.0700	0.0779	0.0744

Females

	Age (In years)					
	16-19	20-29	30-39	40-49	50+	16-68
c	1.1549	1.1599	1.1423	1.1333	1.1339	1.1567
m	0.0678	0.0717	0.0632	0.0612	0.0645	0.0717

Then, SIRI's formula is used to obtain the percentage of fat.

POLLOCK et al. (1984) found another system of equations for each sex, in which the estimation is based not only on skinfolds, but also on other measurements such as knee diameter and even breast size in women.

JOHNSTON (1982) developed a formula to predict fat weight in adolescent people, based on a multiple linear relation between B.M.I., tricipital skinfold and age:

Girls : %Fat = 0.355 x age + 1.109 x B.M.I. + 0.170 x tricipital skinfold - 15.869

Boys : %Fat = 0.492 x age + 0.584 x B.M.I. + 0.668 x tricipital skinfold - 1.024

This model is useful only for populations similar to the one on which the model is based.

In 1987, WELTMAN made estimations for fat people, whose skinfold

measurements are not as precise as desirable. This author derived a series of equations as follows:

Women: kg of fat = 0.46361 x CG + 0.31303 x CMA + 0.13614 x height + 0.22885 x CM - 83.43495

Men: kg of fat = 0.46590 x CMA + 0.20468 x CM - 39.642

where CMA is the mid-abdomen circumference, CM is the thigh circumference and CG is the gluteus circumference.

Circumferences have evidently been chosen due to the imprecisions in measurements of skinfolds thicker than 40-50 mm, though circumferences are more associated with lean tissue than with fat tissue. The prediction error is lower than 4% in females and 3% in males.

Hydrometry

Every method used to establish the total body water is divided into two separate fractions (extra - and intracellular water). This determination is the basis estimation of the water-free mass.

Various substances are used in this technique:

- antipyrine
- urea
- heavy water (DHO)
- tritiated water (THO)
- sulphamide

The extracellular composition is determined by using thiocyanate and correcting with a factor of 0.7 to obtain the real extracellular space.

Intracell. water = total water - extracell. water

According to BROŽEK (1961):

$M = F + N$

where M = total body weight, F = fat weight and N = everything that is not fat.

$N = T - K$

where T is the total body water and K is a constant that, in adult humans, ranges from 0.71 to 0.73.

In a study on body water, SHENG and HUGGING (1979) established that the fat-free weight contains about 73.2% of water. This is of great importance because it allows separation of the body mass into two compartments.

For example, if a man weighs 70 kg, with K= 0.732, the percentage of fat is 15%.

Isotope radiometry

In 1953, FORBES et al. discovered that the lean body mass contains more potassium that the rest of the body. About 0.0019% of the total potassium in the organism is radioactive potassium (K40), and so it is possible with a radioactivity counter to evaluate the total potassium content of the body and then, the lean body weight, as follows:

$$\text{Lean body weight} = \frac{\text{total potassium (g)}}{2.66271}$$

given that in adults there is a content of 68.1 meq/kg and that 1 meq weighs 0.0391 g.

In 1963, FORBES also estimated that:

$$\text{Fat weight} = \frac{\text{total body weight - total potassium}}{68.1}$$

LUKASKI et al. (1981) compared the results of calculating the lean body weight and the percentage of fat by several methods and found that these results were coincident:

	Densitometry	K40	Body water
Lean body weight	62.3	62.2	63.9
% Fat	15.2	15.5	13.9

The radioactive potassium method is not traumatic and does not need large equipment as hydrometry does, but it is not a very reliable method to use on very thin individuals. In middle-aged women, there is also an overestimation of the fat tissue when this technique is used (NOPPA et al., 1979). FOMON et al. (1982) pointed out that an overestimation of about 13% was observed when they applied the radioactive potassium method to children aged 9-10 years.

Computerized axial tomography

This method can be applied from photometry used to study densitometry and bone composition. There are actually three basic techniques to determine bone mass which are not "invasive":

1. Photometric absortion of a simple photon.
2. Photometric absortion of a double photon.
3. Computerized tomography.

The first method can be used to determine the peripheral skeleton bone tissue at certain points of the osseus cutting. This technique is fast, cheap and uses low radiation, and it is possible to study trabecular bone areas with it.

The second method is a logical evolution of the first one. It allows characterization of the whole skeleton and not only of one area. This method needs more time, but the doses of radiation are lower than in the first one and it is also highly precise.

Tomography allows the direct measurement of the trabecular bone density, mainly at the vertebral column level. The advances in computerization and software are continuously improving explorations, comparisons and diagnosis.

Thus, in individuals suffering from severe obesity, tomography is a good estimation method. Starting from bone standards, which must be corrected to fit each population, with the latest generation of densitometers it is possible to know the body composition of these individuals compared to the references and according to their

sex, race, height, weight, etc.

This method has permitted the correction of some estimation formulae on body composition.

The study of adipocytes

Adipocyte size and number can also be included in body composition studies. There are some specialized techniques, such as direct measurements of frozen cells obtained by surgical incisions. Adipocyte size is estimated from a very small portion of subcutaneous fat ($1/10^6$), the result being extrapolated to the rest of the fat stores, though some differences in size have been found depending on the part of the body where the adipocytes are from and the age of the individual. Thus, for example, bigger sizes were found in the abdominal wall and gluteus. These differences are larger in children than in adults.

With regard to the ontogenetic process, it has been observed that the number of adipocytes mainly increases during the first year of life, though at the age of 4 years there is another slight increase in number. In adults there are no appreciable changes.

Nevertheless, the increase in size can vary in relation to metabolic alterations or obesity, mainly in the abdominal adipocytes.

High correlations with the fat fraction of the body composition have also been observed.

Applications

I should like to talk to you now about one application of body composition. These investigations find great application in the field of health and the determination of the state of human nutrition.

The study analyses the changes in body composition and the increase and distribution of fat tissue in women suffering from severe undernutrition due to starvation, with a great loss of muscular tissue. Our main goal in this first study was to know the somatic moment at which these women re-started their menses, bringing new data to the theory of the threshold in the percentage of fat needed to start and maintain the menstrual function.

In 1990, a group of prisoners belonging to a terrorist group operating in Spain (GRAPO) began a hunger strike to pressure the authorities into making a series of concessions. The strike began on November 26, 1989, and ended on February 8, 1991. Not all members of the group upheld it with the same degree of firmness; only a minority did so.

Two days after the strike ended, the medical department of the Women's Penitentiary Centre in Madrid commissioned us to carry out somatic and physiological studies of two female prisoners, M. and V. They had been transferred to this prison one year before, both in a state of considerable deterioration. The principal disorder they were diagnosed as suffering from was a type of encephalopathy known as

Wernike's disease.

These women had adopted a rigid stance about eating: they agreed to be fed intravenously with a glucose solution (500 cm^3 3 times a day) and a glucosaline solution (500 cm^3 2 times a day). After 15 months on the hunger strike, the loss of muscle tissue was dramatic and they were scarcely able to hold themselves erect.

Measurements were made every two weeks. Data were gathered according to I.B.P. norms, but their severely incapacitated state demanded that outside assistance was required. We considered height, weight, circumferences (arm, waist and hip), skinfolds (tricipital, bicipital, subscapular and suprailiac) and general capacity. These women, who were gynaecologically controlled, obviously suffered from amenorrhoea.

Tables 1 and 2 show somatic measurements on M. and V. Their height was 154.3 and 160 cm, respectively. When my study began, this values the weights were below the 10th and 5th percentiles for their sex and age.

Table 1. Somatic measurements on men.

TRI.	BI.	SUP.	SUB.	ARM.	WAI.	HIP.	V.C.	WEI
05.6	5.0	5.4	5.2	19.9	60.0	72.6	2.5	35.0
07.8	6.2	6.2	5.2	21.0	63.0	81.0	2.8	39.0
08.2	7.0	7.4	6.8	21.2	63.9	81.0	2.4	43.0
09.8	5.0	9.8	7.8	22.4	65.8	82.3	2.5	42.0
10.0	6.0	9.0	7.8	22.5	66.4	82.1	2.2	43.0
11.8	7.2	9.2	7.8	22.4	67.4	82.2	2.1	44.5
09.8	6.8	12.4	6.6	24.4	67.2	82.4	2.6	46.0
10.8	8.6	13.6	8.4	25.0	67.2	82.9	2.8	46.5
14.8	9.0	13.6	9.2	25.0	67.4	84.5	2.7	46.5
15.6	9.0	12.6	9.4	25.5	70.0	87.0	3.1	48.4

Table 2. Somatic measurements on women.

TRI.	BI.	SUP.	SUB.	ARM.	WAI.	HIP.	V.C.	WEI
06.8	6.2	6.4	5.2	19.2	65.2	75.3	1.8	39.5
11.0	7.2	7.6	6.8	20.1	69.3	80.1	2.5	43.0
12.6	7.8	8.2	7.8	22.1	70.1	84.6	2.6	49.0
15.0	8.8	8.6	10.0	23.8	71.7	88.0	2.8	56.4
15.3	9.2	8.9	10.1	24.0	72.3	88.2	2.7	56.4
16.6	9.4	9.0	10.2	22.4	73.3	88.4	2.7	56.0
16.8	9.4	9.8	10.3	22.4	74.0	89.1	2.6	55.0
16.2	9.4	8.4	10.8	25.8	74.3	89.9	3.4	56.0
19.8	10.2	10.6	8.8	26.0	74.5	89.0	3.5	56.0
19.8	9.8	10.8	9.8	26.6	75.6	88.2	2.5	57.0

As conserns nutrition, the medical team gradually modified the women's diet and their level of physical activity (Table 3).

The day after these measurements were taken, the women had just begun to receive a very light diet. The estimated percentage of fatty tissue was about 15%, and the density was relatively high. Complete bed rest was prescribed during this period. The women performed no physical activity at all. A complex "Pentaplus", with

vitamins and minerals, was provided to the women every day.

Table 3. Daily nutrition.

BABY FOOD	PROT.	LIP.	C.H	Kcal	
Chicken/rice		6.6	2.2	19	124
Chicken/calf		6.6	3.2	12	102
Hake/vegetable	7.0	0.4	18.2	104	
Fruit (100g)		0.3	0.1	17.7	73
PENTAPLUS (1) 200 ml	19.4	4.2	20.8	200	
FURICH (3)				780	
TOTALS	39.9	10.3	87.7	1,383	

Over a period of 5 months, the somatic parameters changed favourably: M. gained 13.4 kg and V. gained 17.5 kg. This gain in weight involved a replacement of the fat reserves in the skinfolds. The percentage of fat in both women was higher than 26%. Due to the women's rapid weight gain, the medical team put them on a more controlled diet during the second half of March, when the recovery was well established.

If we take the ratios of body mass and waist and hip measurements as indicators of the distribution of recently acquired fat (Table 4), we can see a constant increase in both women. The fact that the waist/hip ratio increases could indicate a tendency to fat accumulation around the waist. This always contitutes a greater risk factor for some cardiovascular diseases, according to VAGUE et al. (1988). On the other hand, the robusticity index rose.

Table 4. ROHRER index, waist/hip ratio, and Quetelet index.

	MEN	
R.I.	W/H	Q.I.
09.46	0.82	152.38
10.30	0.77	165.88
11.74	0.78	189.03
13.51	0.79	217.58
13.51	0.80	217.58
13.51	0.81	216.04
13.17	0.81	212.18
13.17	0.81	218.18
13.41	0.82	216.04
13.65	0.83	219.83

	WOMEN	
R.I.	W/H	Q.I.
9.69	0.86	148.73
10.80	0.86	165.73
11.91	0.82	182.73
11.63	0.81	178.48
11.91	0.81	182.73
12.32	0.82	189.10
12.78	0.83	195.48
12.88	0.82	197.60
12.88	0.83	197.60
13.40	0.85	205.68

What happened with the gynaecological history? These were studied women in order to relate the menstrual cycle to the body composition (Table 5). Before the hunger strike began, the women had not experienced any periods of amenorrhoea or serious irregularities. Menarche was 12.5 for M. and 13.0 for V. After the strike was under way, the women had lost 20% of their normal weight in December (the strike began in November).

Table 5. Somatic parameters after menstruation began again.

	V.	M.
WEIGHT	49kg	45.5kg
SKINFOLDS	36.4 mm	44.2 mm
DENSITY	.040	1.038
% FAT	24.6 %	26.6%
F.B.W/L.B.W	3.06	2.78
QUATELET INDEX	182.73 g/cm^2	216.08 g/cm^2
ROHRER INDEX	11.91 kg/m^3	13.41 kg/m^3
	DATE: MARCH '91 AMENORRHOEA: 15 M.	DATE: MAY '91 AMENORRHOEA: 17 M.

After the hunger strike was over and the women began to eat normally, they began to menstruate, V. in March and M. in May.

We should point out that the women's periods of ammenorrhoea did not have the same duration. As regards the changes in their body composition, it can be seen in Table 5 that the percentage of body fat was 25%, and one of the most important things: the density was more or less the same for both.

When we compare the changes in the body fat proportions in these women with the monograms of MCARTHUR, we can see that, for height, the weight and percentage of fat are very similar to those indicated for the renewal of menstruation. Our women needed more than 22.0% for the renewal of menstruation, perhaps because of the extreme wasting of the body caused by the prolonged hunger strike.

I must end now, I hope that you understand more than at the beginning of my contribution as concerns the important relation of body composition and fertility in women.

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METHODOLOGICAL NOTES ON TWO CROSS-SECTIONAL GROWTH STUDIES

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Abstract

Comparisons were made of the body height and weight parameters established in two large cross-sectional growth studies. These separate studies reveal common features, but also differences. The unpaired t-test demonstrated the following significant differences: among the 32 age groups (created by dividing the core samples into 6-month age groups ranging from 3 to 18 years of age), significant differences in height appeared in 15 cases among the boys and in 9 cases among the girls. As regards the weights, 14 of the age groups among the boys and 25 of the age groups among the girls revealed significant differences. These constitute 49.2% of the total cases.

This comparison draws attention to the importance of the reliability of measuring and the choice of anthropological sampling.

Key words: growth standards, Hungary

Introduction

National standards are essential in monitoring the physical condition of children. All ethnic groups differ from others in taxonomic, ethnogenetic, historical and other aspects. In establishing the body development of the young of a certain ethnic group, therefore the most appropriate standards are those derived from the somatometric data of the same ethnic group.

For many years, American standards served as the benchmark in Hungary. Although sporadic local growth studies have been carried out in Hungary, these were not suitable for setting up national growth standards for the following reasons:

- the measurements were made by different persons,
- the investigations were not systematic and intensive,
- the age groupings differed in the various studies.

Growth studies must comply with basic requirements. If the data are to be used as a reference, care must be taken to ensure representative and random sampling. The

evaluations must also follow standardization. Among the Hungarian growth studies, the largest difference usually appears in the age grouping.

These facts led the Department of Anthropology in Budapest to organize a nation-wide cross-sectional sampling under the supervision of OTTÓ EIBEN. The investigation was designed to yield information on the physical status and body development of Hungarian children as a function of the influence of the socialist society:

- within the frame of the socialist social structure up to the 1980s;
- under the conditions of urbanization due to socialist industrialization;
- under the influence of the class restructuring of the population during the 30 years of the socialist society;
- within the bounds of urban and rural ways of life (EIBEN and PANTÓ, 1981).

Since the time of those studies, it has emerged that the achievement of a truly socialist society in Hungary appears to be merely a utopia, and secularization of the young can be attained even without socialism.

Data collection extended throughout Hungary and involved 1.5-2% of the population in the age range 3-18 years (EIBEN, 1986).

In consequence of the uncoordinated growth studies in Hungary, the Department of Anthropology in Szeged also organized such a project at the beginning of the 1980s, under the supervision of GYULA FARKAS (FARKAS, 1990). This was aimed at investigating the relationship between the menarche and the socio-economic and physical factors, but the height, weight and normal chest circumference were also studied.

Material and method

As the two projects were virtually simultaneous, their comparison seems obvious.

In the remainder of this paper, the research carried out at the Department of Anthropology at Lóránd Eötvös University will be referred to as NGS, the abbreviation for National Growth Standard, while the investigation carried out by the Department of Anthropology at Attila József University will be referred to by the abbreviation Sz, for Szeged.

The NGS and Sz samples share the following features:

- both samplings compiled data on Hungarian children;
- the data collections were carried out at the beginning of the 1980s;
- both boys and girls were measured, in age groups ranging from 3 to 18.5 years;
- the samples were comprehensive;
- the measurements followed the standards set by Martin;
- the evaluations were carried out by computer in 6-month age groups;
- the measurements were made by different persons.

The samples differ in the following ways:

- NGS sampling is a proportional one, taking into consideration the total numbers of boys and girls in a given county, while half of the total Sz sample is from Csongrád County, representing 95% of the 3-18-year-old boys and girls living in that county; the remainder of the sample was collected by random sampling from the other 18 counties of the country;
- NGS measurements were recorded by different persons; in the case of the Sz sample, specific measurements were always made by the same person with the same instrument;
- NGS used a spring scale to measure the weight with 500 gram accuracy (BARABÁS et al., 1990), whereas Sz used a medical scale with 50 gram accuracy;

- normal NGS values were given in percentile, whereas Sz gave an interval of $x \pm 1.96$ s;
- the difference in the sample size is significant with regard to the girls, but less so for the boys;
- the sample sizes differ in the specific age groups.

The present study was set up to elucidate whether or not there are differences between the two samples with regard to the mean measurements for the various age categories. Boys and girls were evaluated separately.

Normal chest circumference was not comparable, as the age groupings in the two samples were dissimilar. The appropriate pairs of means were tested by the t-test.

Results

Parameters of height and weight are shown in Tables 1-4, where the means, the sample sizes and the standard deviations for the specific age groups of the NGS study are denoted by number 1, and those of the Sz study by number 2. The difference between the means of the samples in relation to age is denoted by "d", "t" is the result of the t-test and "P" signifies the level of significance.

Results are summarized in Table 5.

This Table illustrates that, as concerns the 32 age groups, created by dividing the core samples into 6-month age groups from 3 to 18 years of age:

1. the Sz sample indicated that the height was greater in 24 cases among the boys and in 25 cases among the girls;
2. these differences were significant in 15 age groups among the boys and in 9 age groups among the girls;
3. 5 of the above significant deviations were greater for the Sz sample, and 10 for the NGS sample; among the 9 significant deviations of the heights of the girls, 1 was greater for the Sz sample, and 8 for the NGS sample.
4. With regard to the weight, 24 of the age group data for the boys and 31 of those for the girls were greater in the Sz sample.
5. Of the differences in point 4, these were significant in 14 of the age groups among the boys, and 25 of the age groups among the girls.
6. 13 of the 14 significant differences in the measurements on the boys were greater in the Sz sample and 1 in the NGS sample. As concerns the measurements on the girls all the 25 significantly different age group values were greater in the Sz sample.
7. Among the 63 significant differences, 12 were significant at the 0.005 level, and 51 at a level less than 0.05.

Table 1. Parameters relating to boys' heights in 6-month age groups.

Age	$\overline{x}_1$	$\overline{x}_2$	d	n_1	n_2	s_1	s_2	S	t	P
3	98.13	96.24	1.89	65	98	3.70	3.97	3.86	3.06	**1**
3.5	99.59	99.39	0.20	101	310	4.37	4.20	4.24	0.41	ns
4	103.79	102.80	0.99	94	394	4.37	4.36	4.36	1.98	**5**
4.5	107.23	106.14	1.09	109	518	7.05	4.43	4.98	2.08	**5**
5	110.09	109.01	1.08	122	492	5.25	4.84	4.92	2.17	**5**
5.5	112.48	112.71	-0.23	137	543	4.46	4.88	4.80	0.50	ns
6	116.42	116.34	0.08	151	615	4.18	5.30	5.10	0.17	ns
6.5	117.84	119.07	-1.23	191	599	4.97	5.51	5.38	2.75	**1**
7	121.28	122.21	-0.93	214	688	5.79	5.34	5.45	2.18	**5**
7.5	124.66	125.00	-0.34	184	717	5.40	5.49	5.47	0.75	ns
8	127.29	127.56	-0.27	198	658	5.14	5.37	5.32	0.60	ns
8.5	130.09	130.62	-0.53	188	693	5.95	5.69	5.75	1.12	ns
9	132.02	133.24	-1.22	194	695	6.04	6.21	6.17	2.43	**2**
9.5	134.85	136.03	-1.18	189	719	6.52	6.42	6.44	2.24	**5**
10	138.04	139.06	-1.02	403	713	6.14	6.32	6.26	2.62	**1**
10.5	141.14	140.39	0.75	1164	693	6.26	6.25	6.25	2.50	**2**
11	143.07	143.30	-0.27	1563	718	6.54	6.75	6.61	0.77	ns
11.5	145.71	146.18	-0.47	1636	658	6.78	7.12	6.88	1.48	ns
12	148.57	148.95	-0.38	1663	666	7.41	7.11	7.33	1.13	ns
12.5	151.22	152.34	-1.12	1663	700	7.91	8.23	8.01	3.11	**0.2**
13	155.11	155.67	-0.56	1735	711	8.32	8.26	8.30	1.52	ns
13.5	158.40	158.87	-0.47	1711	706	8.51	8.53	8.52	1.23	ns
14	162.19	162.60	-0.41	1585	747	8.62	8.38	8.54	1.08	ns
14.5	165.08	166.16	-1.08	1516	774	8.58	8.43	8.53	2.87	**1**
15	167.83	168.72	-0.89	1158	889	7.99	7.90	7.95	2.51	**2**
15.5	170.51	171.40	-0.89	1095	847	7.27	7.38	7.32	2.66	**1**
16	171.63	172.19	-0.55	1021	852	7.06	6.76	6.93	1.74	ns
16.5	173.21	173.41	-0.20	905	796	6.64	6.66	6.65	0.61	ns
17	173.43	174.41	-0.98	834	758	6.68	7.10	6.88	2.84	**1**
17.5	174.39	174.88	-0.49	525	596	6.47	6.58	6.53	1.25	ns
18	175.48	175.58	-0.10	408	436	6.86	6.87	6.86	0.21	ns
18.5	174.56	172.10	2.46	126	244	6.65	15.35	13.06	1.72	ns
sum.				22848	20243					

Note: $\mathbf{x_1}$, $\mathbf{n_1}$ and $\mathbf{s_1}$ are the parameters of the Sz sample, while $\mathbf{x_2}$, $\mathbf{n_2}$ and $\mathbf{s_2}$ are those of the NGS sample, **d** is the deviation between x_1 and x_2, **S** is the common standard deviation, **t** is the result of the t-test, and **P** is the level of significance.

Table 2. Parameters relating to girls' heights in 6-month age groups.

Age	$\overline{x}_1$	$\overline{x}_2$	d	n_1	n_2	s_1	s_2	S	t	P
3	97.01	95.99	1.02	57	118	3.86	3.67	3.73	1.69	ns
3.5	98.52	98.95	-0.43	93	317	3.82	3.93	3.91	0.93	ns
4	103.35	101.76	1.59	112	441	3.95	4.46	4.36	3.44	**0.1**
4.5	105.75	105.53	0.22	118	469	3.89	4.58	4.45	0.48	ns
5	109.83	109.18	0.65	135	497	4.53	4.82	4.76	1.41	ns
5.5	113.16	112.67	0.49	136	565	4.57	4.82	4.77	1.07	ns
6	115.76	115.97	-0.21	152	629	5.24	5.25	5.24	0.44	ns
6.5	117.28	118.72	-1.44	188	626	5.15	5.30	5.27	3.29	**0.1**
7	120.99	121.63	-0.64	187	579	5.77	5.15	5.31	1.43	ns
7.5	123.62	124.16	-0.54	184	624	5.10	5.71	5.58	1.15	ns
8	126.18	127.32	-1.14	209	648	5.14	5.72	5.58	2.57	**2**
8.5	129.11	129.65	-0.54	187	715	6.32	5.84	5.94	1.11	ns
9	131.96	132.67	-0.71	215	680	6.91	6.28	6.44	1.41	ns
9.5	134.79	135.24	-0.45	207	642	6.65	6.15	6.28	0.90	ns
10	138.82	138.13	0.69	414	622	6.86	6.60	6.71	1.62	ns
10.5	141.52	141.58	-0.06	1444	663	6.78	6.83	6.80	0.19	ns
11	144.45	144.72	-0.27	1871	693	7.04	7.17	7.08	0.86	ns
11.5	147.34	147.49	-0.15	2041	674	7.35	6.98	7.26	0.47	ns
12	150.67	150.81	-0.14	1920	680	7.27	7.62	7.36	0.43	ns
12.5	153.50	153.89	-0.39	2034	710	6.94	6.99	6.95	1.29	ns
13	155.79	155.89	-0.10	2031	685	6.75	6.95	6.80	0.33	ns
13.5	157.76	157.97	-0.21	2004	665	6.50	6.60	6.53	0.72	ns
14	159.13	159.23	-0.10	1954	655	6.17	6.26	6.19	0.36	ns
14.5	159.77	160.60	-0.83	1972	750	6.01	6.35	6.11	3.17	**0.2**
15	160.75	161.28	-0.53	2137	789	6.05	6.37	6.14	2.07	**5**
15.5	160.95	161.84	-0.89	1905	662	5.97	6.24	6.04	3.27	**0.2**
16	161.14	161.88	-0.74	1690	723	5.94	5.99	5.96	2.80	**1**
16.5	161.39	161.95	-0.56	1453	683	6.36	5.78	6.18	1.95	**5**
17	161.62	162.28	-0.66	1362	630	6.15	5.98	6.10	2.25	**5**
17.5	161.84	161.95	-0.11	1074	508	6.19	6.03	6.14	0.03	ns
18	162.03	162.45	-0.42	836	427	5.83	5.93	5.86	1.20	ns
18.5	161.36	160.12	1.24	253	199	6.33	13.23	9.97	1.31	ns
sum.				30575	18968					

Note: $\mathbf{x_1}$, $\mathbf{n_1}$ and $\mathbf{s_1}$ are the parameters of the Sz sample, while $\mathbf{x_2}$, $\mathbf{n_2}$ and $\mathbf{s_2}$ are those of the NGS sample, **d** is the deviation between x_1 and x_2, S is the common standard deviation, **t** is the result of the t-test, and **P** is the level of significance.

Table 3. Parameters relating to boys' weights in 6-month age groups.

Age	$\overline{x}_1$	$\overline{x}_2$	d	n_1	n_2	s_1	s_2	S	t	P
3	15.72	14.57	1.15	65	98	1.57	1.70	1.65	4.36	**0.1**
3.5	15.77	15.40	0.37	101	310	1.73	2.02	1.95	1.65	**ns**
4	17.48	16.10	1.38	94	394	2.41	1.93	2.03	5.92	**0.1**
4.5	18.27	17.00	1.27	109	518	2.19	2.19	2.19	5.50	**0.1**
5	19.13	17.89	1.24	122	492	2.87	2.53	2.60	4.71	**0.1**
5.5	19.91	19.13	0.78	137	543	2.53	2.86	2.80	2.92	**1**
6	21.36	20.54	0.82	151	615	2.85	3.26	3.19	2.85	**1**
6.5	21.89	21.53	0.36	191	599	2.98	3.52	3.40	1.28	**ns**
7	23.66	22.63	1.03	214	688	4.17	3.64	3.77	3.49	**0.1**
7.5	24.69	24.11	0.58	184	717	3.65	4.05	3.97	1.77	**ns**
8	26.33	25.40	0.93	198	658	4.24	4.40	4.36	2.63	**1**
8.5	27.79	27.31	0.48	188	693	4.47	5.18	5.04	1.16	**ns**
9	28.69	28.49	0.20	194	695	5.06	5.64	5.52	0.45	**ns**
9.5	30.76	30.46	0.30	189	719	5.56	6.48	6.30	0.58	**ns**
10	32.90	32.51	0.39	403	713	6.46	6.83	6.70	0.93	**ns**
10.5	34.89	33.13	1.76	1165	693	7.21	6.41	6.92	5.30	**0.1**
11	36.34	35.44	0.90	1563	718	7.41	7.57	7.46	2.68	**1**
11.5	38.26	37.35	0.91	1636	658	8.10	8.51	8.22	2.40	**2**
12	40.50	39.59	0.91	1663	666	8.72	8.98	8.80	2.26	**5**
12.5	42.52	42.15	0.37	1663	700	9.48	9.89	9.60	0.86	**ns**
13	45.74	44.53	1.22	1735	711	10.06	10.01	10.05	2.71	**1**
13.5	48.17	47.61	0.56	1711	706	10.49	10.17	10.40	1.20	**ns**
14	51.86	50.97	0.89	1585	747	11.01	10.54	10.86	1.85	**ns**
14.5	54.26	55.27	-1.01	1516	774	10.82	11.50	11.05	2.07	**5**
15	57.08	57.72	-0.64	1158	889	10.60	10.69	10.64	1.35	**ns**
15.5	59.65	60.56	-0.91	1095	847	10.55	10.28	10.43	1.91	**ns**
16	61.58	62.33	-0.75	1021	852	10.08	10.69	10.36	1.56	**ns**
16.5	64.07	64.34	-0.27	905	796	10.94	10.04	10.53	0.53	**ns**
17	64.92	65.46	-0.54	834	758	10.13	9.57	9.87	1.70	**ns**
17.5	65.72	66.65	-0.93	525	596	9.79	9.93	9.86	1.58	**ns**
18	67.33	67.56	-0.23	408	436	9.61	9.77	9.69	0.34	**ns**
18.5	65.64	65.05	0.59	126	244	8.89	13.71	12.29	0.44	**ns**
sum.				22849	20243					

Note: $\mathbf{x_1}$, $\mathbf{n_1}$ and $\mathbf{s_1}$ are the parameters of the Sz sample, while $\mathbf{x_2}$, $\mathbf{n_2}$ and $\mathbf{s_2}$ are those of the NGS sample, **d** is the deviation between x_1 and x_2, S is the common standard deviation, **t** is the result of the t-test, and **P** is the level of significance.

Table 4. Parameters relating to girls' weights in 6-month age groups.

Age	$\overline{x}_1$	$\overline{x}_2$	d	n_1	n_2	s_1	s_2	S	t	P
3	15.51	14.06	1.45	57	118	2.28	1.73	1.93	4.67	**0.1**
3.5	15.42	14.90	0.52	93	317	1.72	1.92	1.88	2.35	**2**
4	17.00	15.54	1.46	112	441	2.37	2.13	2.18	6.32	**0.1**
4.5	17.63	16.60	1.03	118	469	2.41	2.25	2.28	4.38	**0.1**
5	19.06	17.96	1.10	135	497	2.91	2.52	2.61	4.35	**0.1**
5.5	20.33	19.30	1.03	136	565	3.12	3.01	3.03	3.56	**0.1**
6	21.29	20.44	0.85	152	629	3.37	3.59	3.55	2.65	**1**
6.5	21.23	21.27	-0.04	188	626	4.46	3.63	3.84	0.13	ns
7	23.12	22.56	0.56	187	579	3.14	3.87	3.71	1.80	ns
7.5	24.48	23.60	0.88	184	624	4.21	4.49	4.43	2.37	**2**
8	25.42	25.02	0.40	209	648	4.49	4.44	4.45	1.13	ns
8.5	27.13	26.42	0.71	187	715	4.93	5.05	5.03	1.72	ns
9	28.61	28.52	0.09	215	680	5.83	5.67	5.71	0.20	ns
9.5	30.94	29.88	1.06	207	642	7.00	6.44	6.58	2.02	**5**
10	33.19	31.29	1.90	415	622	7.62	6.32	6.87	4.36	**0.1**
10.5	35.32	33.84	1.48	1444	663	7.51	7.57	7.53	4.19	**0.1**
11	37.29	36.40	0.89	1871	693	8.19	8.05	8.15	2.45	**2**
11.5	39.63	37.73	1.90	2040	674	9.09	7.98	8.83	4.84	**0.1**
12	42.58	41.03	1.55	1920	680	9.25	9.41	9.29	3.74	**0.1**
12.5	45.40	43.81	1.59	2035	710	9.67	9.30	9.58	3.81	**0.1**
13	47.04	47.03	0.01	2030	685	9.04	9.74	9.22	0.03	ns
13.5	49.72	48.23	1.49	2004	665	9.73	9.57	9.69	3.44	**0.1**
14	51.09	50.10	0.99	1953	655	8.95	9.07	8.98	2.44	**2**
14.5	53.15	51.33	1.82	1973	750	9.14	8.39	8.94	4.75	**0.1**
15	54.34	53.25	1.09	2129	789	8.74	8.85	8.77	2.98	**1**
15.5	54.89	54.07	0.82	1901	662	8.28	8.74	8.40	2.16	**5**
16	55.55	54.23	1.32	1690	723	8.31	8.13	8.26	3.60	**0.1**
16.5	55.93	55.00	0.93	1453	683	8.10	8.30	8.16	2.46	**2**
17	56.27	54.77	1.50	1362	630	7.90	8.97	8.25	3.77	**0.1**
17.5	56.43	54.61	1.82	1072	508	8.00	7.89	7.96	4.24	**0.1**
18	56.44	55.70	0.74	836	427	8.10	9.25	8.51	1.46	ns
18.5	55.81	52.92	2.89	253	199	7.93	10.79	9.30	3.28	**0.2**
össz.				30561	18968					

Note: $\mathbf{x_1}$, $\mathbf{n_1}$ and $\mathbf{s_1}$ are the parameters of the Sz sample, while $\mathbf{x_2}$, $\mathbf{n_2}$ and $\mathbf{s_2}$ are those of the NGS sample, **d** is the deviation between x_1 and x_2, **S** is the common standard deviation, **t** is the result of the t-test, and **P** is the level of significance.

Table 5. Numbers of deviations between the examined samples.

Averages of the two samples	Height		Weight	
	Boys	Girls	Boys	Girls
Sz > NGS	8	7	24	1
not significant	17	23	18	2
significant	15	9	17	25

Discussion

These results raise the question of the cause of these deviations between the NGS and Sz samples.

Our first suggestion is the difference in the sampling techniques. This could be proved if the deviations were consistent. Table 5 shows that the NGS means are higher for both sexes as far as the height is concerned and also for the girls' weight, but for the boys' weight the Sz means are higher. Therefore, although different sampling technique may cause differences, this is not the sole reason.

The different sample sizes of the various age groups can also lead to significant differences, but the inconsistent deviations contradict this reasoning. This is illustrated by the data in Table 2, where the sample sizes of the 6-9.5-year age groups are larger in the Sz sample, whereas the 10.5-18-year age groups are greater in the NGS sample; however, in all of these age groups, the means are lower in the Sz sample.

Different techniques in scaling body weight could be another cause of the differences, but in the case of the boys higher values of weight can be observed in the Sz sample, while in the case of girls the NGS weight means are larger. The only consistent deviations appear in the heights and weights of both boys and girls in the 3-5-year age ranges, with the exception of the girls' height in the 3.5-year age category, in which the Sz means are higher in 20 cases, 14 of them significantly so.

As the weight is low at these early ages, the scaling technique could lead to different results as NGS operated with 500 gram accuracy, while Sz used a scale with 50 gram accuracy.

In conclusion, the above-mentioned facts do not satisfactorily explain the differences, though these constitute 49.2% of the total cases.

It can be mentioned that the menarche medians are fully equivalent, with a median age of 12.79 years. The evaluations (probit analysis) followed different procedures and even the different sample sizes could lead to different results.

This question can be settled only with a new sampling with a standardized procedure, standardized measurements and standardized age grouping.

We conclude that the deviations between these two large studies raise the question of the selection of the sampling technique. Even if the most appropriate technique is chosen, many factors can influence the success, such as financial background, the time of the sampling, the number of people on the staff, etc.

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LOBODONTIA
Literature review and case report

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Abstract

A case of lobodontia is described which involves anomalies of shape, size, number, eruption, and positions of the teeth. The hereditary line is autosomal dominant, not X-linked. The esthetic problems and secondary diseases were treated.

Key words: lobodontia, dental anomalies, case report.

Introduction

Lobodontia is a set of simultaneous anomalies in shape, size, number, eruption, position, and structure of the teeth. This anomaly occurs without other anomalies or phenomena of any syndrome (ROBBINS and KEENE, 1964; SHUFF, 1972).

Literature review

The anomaly was first described in detail by ROBBINS and KEENE (1964). However, there is a description of a case together with some drawings from the end of the 19th century (ROBBINS, 1890, cit. in COLYER, 1910). That report presents an early review of lobodontia in the upper and lower dental arches in a patient and his relatives. "In two sisters of this patient a similar condition existed, though not so well marked, and some cousins were also stated to have shown the same peculiarities."

According to BROOK and WINDER (1979), lobodontia was defined by KEENE and DAHLBERG in 1973 as a multiple tooth anomaly resembling the dentition of carnivores (lobos - wolf). This terminology was used by DIXON and STEWART in 1976, too. However, as concerns the interpretation of this technical term, NATHANAIL (1979) and

WALTERS (1980) point out that the Greek "lobos" means "lobe" and "odontia" means "dentition", while the Latin "lobulus" is "lobe" and "lupus" is a "wolf". On this basis:

1. The anomaly may be called "lupusodontia" (NATHANAIL, 1979), or "lupusdentia" (WALTERS, 1980) in Latin, or " lykodontia" in Greek.

2. However, the term "lobodontia" meaning lobed (or lobular) dentition is more correct, in spite of the fact that it concerns only one member of this set of anomalies, namely the tooth form.

In the permanent dentition, the anomaly may affect practically all teeth. The literature data (ROBBINS and KEENE, 1964; SHUFF, 1972, CASAMASSIMO et al, 1978; BROOK and WINDER, 1979) indicate that its characteristic features in the different tooth groups are as follows:

Incisors: marked cingulum formation, invagination, shovel-shaped, accentuated marginal folds, and lobulation.

Canines: extremely pointed and well-developed central lobe, with underdeveloped marginal lobes.

Premolars: well-developed, pointed buccal cusps, and underdeveloped lingual cusps. Teeth with a single root, with possible invagination.

Molars: multitubercular crown form, without the typical groove pattern. Supernumerary, pointed cusps, sometimes occlusally. Single rooted teeth, with pyramidal or taurodont roots.

Beside anomalies concerning the tooth form, hypodontia, delayed tooth eruption, and diminished tooth size are characteristic of this malformation. Positional and structural anomalies may appear, too. In the described cases, no other malformations of the face or of the body were present, from either a physical or a mental aspect.

Lobodontia, as a set of developmental anomalies, may cause secondary pathological lesions through its formal and structural anomalies. BROOK and WINDER (1979) reported on dental invagination causing periodontal pathosis.

The etiology of the anomaly is unknown. It is a hereditary anomaly, the inheritance being autosomally dominant (BROOK and WINDER, 1979). Though not an X-linked anomaly, some authors (CASAMASSIMO et al., 1978) postulate a recessivity linked to the X chromosome, while others (BROOK and WINDER, 1979) consider it to be multifactorially determined, with a polygen component.

KEENE and DAHLBERG (1973) give its prevalence as less than $1:10^6$. The reported cases were Europid persons.

Case reports on lobodontia do not deal with the therapy of the anomaly, but merely with the therapy of the secondary diseases, e.g. extraction of displaced premolars (ROBBINS and KEENE, 1964), or the endodontic therapy of periodontitis caused by invagination (CASAMASSIMO et al., 1978; BROOK and WINDER, 1979).

Case report

Table I. Dental status of patient with lobodontia.

impacted	.	.	.	.	.	germ aplasi	.	.	.	.	.	impacted	.	.	impacted
8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8
erupting	.	extracted	impacted	.	.	.	germ aplasi ?	germ aplasi ?	.	.	.	.	extracted	.	erupting

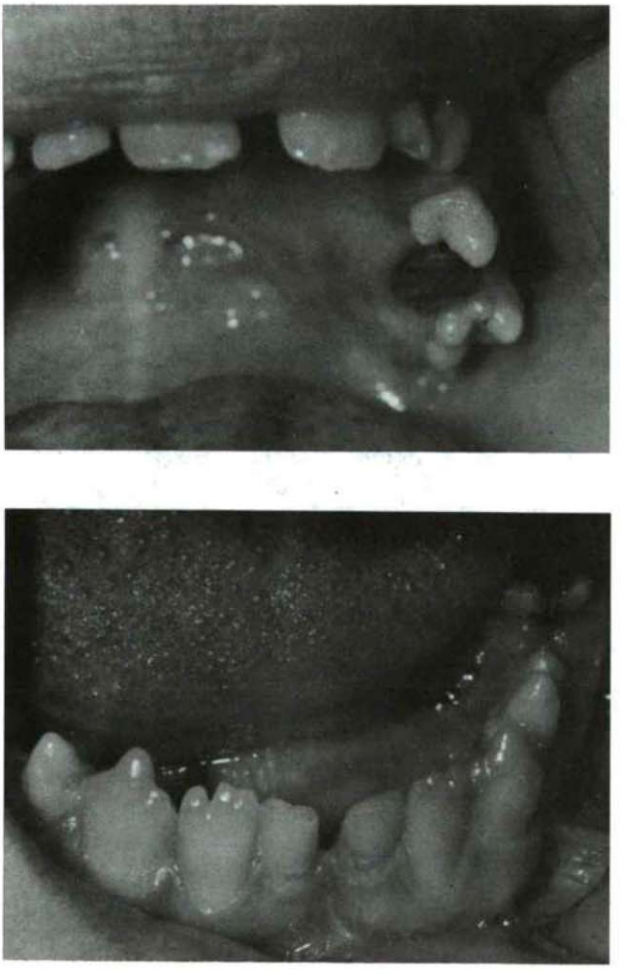

Fig. 1 A, Clinical appearance of the upper dental arch. B, Clinical appearance of the lower dental arch of the lobodontia patient

L.G. a 16-year-old boy, exhibits an unusual form of the teeth (Fig. 1 A,B). The dental status is given in Table 1. The following characteristics of the teeth have been observed:

Incisors: The upper central incisors are slightly barrel-shaped. The incisal edges and the labial surfaces are three-lobed. Throughout the palatinal surface, two parallel grooves run from the incisal edge to the moderately developed cingulum. A moderate tuberculum dentis (talon cusp) can be seen (Fig. 2). Between the two central incisors there is a 2.7 mm diastema (trema). There is aplasia of both upper permanent lateral incisors, with persistence of the deciduous teeth. These reveal a slight shovel-shape.

The two lower central incisors are peg-shaped, with a slightly barrel-shaped form. The incisal edges are worn, and the axis of the crown is slanted distally relative to the root axis (Fig. 1B). Their color resembles that of the permanent teeth. As concerns the extent of the abrasion, however, the persistence of the deciduous teeth and the aplasia of the permanent ones is possible.

The lower lateral incisors are strongly three-lobulated. The distal notch of the incisal edge is so deep that the teeth have a cuspidated appearance. The fissure is marked on the labial and lingual surfaces, too.

Canines: The eruption of the upper cuspids has not been completed yet (Fig. 2). The crowns of these teeth are three-lobulated. The middle lobe is strong and protruding, while the lateral ones are rather low.

The lower cuspids are well-developed, with a less accentuated lobulation than that of the upper ones (Fig. 1B). They are strongly shovel-shaped; the left one is rotated about its long axis.

Premolars: The first upper premolars resemble the canines, or the lower first premolars, having a strong, protruding buccal cusp with a rounded tip. The palatal cusp presents only in the form of a tuberculum dentis. The mesial and distal enamel rims embrace the buccal cusp and form a strong cingulum or palatal talon-cusp (Fig. 1 A). Both upper first premolars present invagination, protruding into the inside of the teeth deeper than the enamel-cemental junction, where the invaginations are dilated. Both teeth are single-rooted, the right one is rotated about its long axis.

Of the upper second premolars, the right one is not completely aligned, while the left one is still in the maxillary bone (retention). The palatal cusp of the right second premolar presents in the form of a medium-sized tuberculum dentis, being divided from the well-developed buccal cusp by a deep groove. There is invagination inside the tooth, in similar proportions as described above. There is bone rarefaction around its apex (Fig. 3). The tooth is rotated about its long axis.

The buccal cusp of the lower first premolars is strong and pointed. There is no invagination inside these teeth.

The lower right second premolar is still in the bone (retention). The left one resembles the first premolar and is erupted. It is also rotated, and presents no invagination.

Fig. 2. Diagnostic models of the upper incisors and cuspids

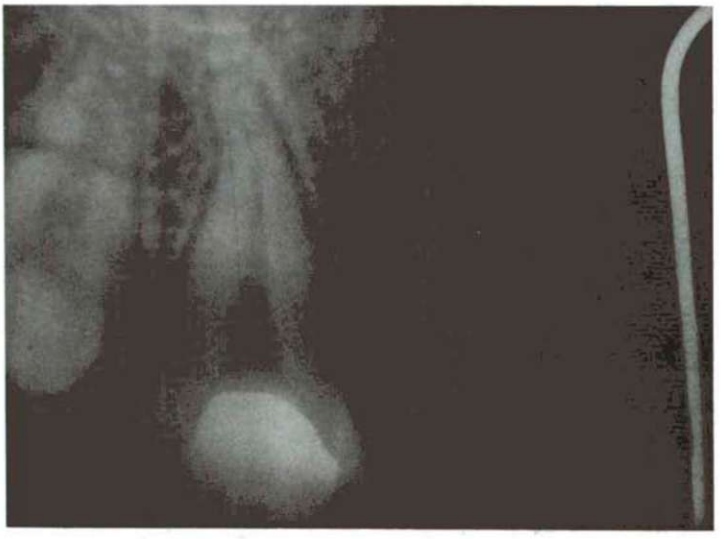

Fig. 3. Intraoral radiograph showing periapical pathosis caused by invagination of the upper right second premolar.

Molars: None of the molars present the usual number of cusps and groove pattern. They have a typical form of 5-7 cusps aligned around a central fovea, without any occlusal cusps, just as in the report by CASAMASSIMO et al. (1978). The first lower molars were extracted a long time ago. The wisdom teeth are still in the bone. The crowns of teeth 16, 26, 37 and 47 are badly destroyed, or filled. All of these teeth, with the exception of the mesotaurodontic lower right second molar (Fig. 4.), are single-rooted, with a pyramidal form.

Fig. 4. Intraoral radiograph showing mesotaurodontic lower right second molar.

The teeth display a reduction in size, similar to the case reported by BROOK and WINDER (1979). Tooth sizes are presented in Table 2.

Table 2. Mesiodistal crown dimensions of patient.

-	8.2	8.8	5.0	5.4	7.0	-	7.5	7.9	-	7.0	5.5	-	-	8.3	-
8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8
-	-	-	-	5.5	6.9	5.0	4.5	4.5	5.0	6.9	5.5	5.8	-	9.6	-

The cheek-bones are well developed, and the TMJ exhibits no functional disturbances. Apart from the tooth anomalies described, no other anomaly or pathological condition is discernible. The patient has a good physique, in accordance with his age. He is a mentally well-developed young man. However, his unusual denture is causing him psychological problems, making him shy and passive.

Information from the father and grandmother of the patient revealed that lobodontia has been present in several generations of the family. The hereditary line of the anomaly is presented in Fig. 5. This indicates that the condition is inherited as an autosomal dominant trait, and is not an X-linked anomaly.

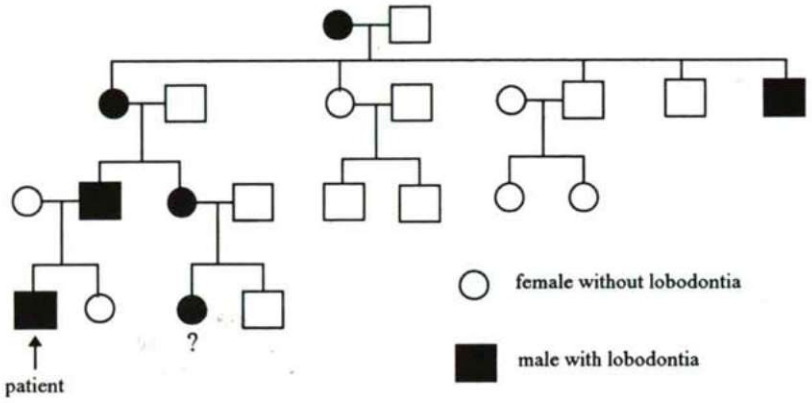

Fig. 5. Pedigree representing an autosomal dominant, and not an X-linked hereditary line.

Fig. 6. The patient with "normalized" tooth form.

We have treated the apical bone rarefaction endodontically, and covered the molar teeth with crowns. The esthetically disadvantageous form anomalies of the front teeth, the cuspidated appearance of the incisal edges, and the large diastema between the upper front teeth have been corrected with composite resin material (Fig. 6).

Discussion

This case is similar to the previously described lobodontia cases. An abnormal tooth form, aplasia of the tooth germ and delayed eruption have been found, similarly as in the literature. Invagination has been found in teeth 14, 15 and 24, whereas the incisors and lower premolars exhibited no such anomaly. In the case of the molars, taurodontia and a pyramidal root form presented concomitantly. The hereditary line has been presented in the form of a table, according to which the lobodontia is inherited dominantly, and is not X-linked.

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ARTHROPATHIES INFLAMMATOIRES DANS LA SÉRIE ANTHROPOLOGIQUE DE SÁRRÉTUDVARI-HÍZÓFÖLD (HONGRIE, XE SIÈCLE AP. J.-C.)

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Résumé

Au cours d'un programme de recherche international, l'étude paléopathologique de la série osseuse de Sárrétudvari-Hízóföld (Est de la Hongrie, période de la Conquête Hongroise) a été réalisée. Outre une série d'altérations pathologiques d'origine diverse (traumatismes, processus dégénératifs, maladies infectieuses, infections locales, processus dystrophiques, trépanations et autres), deux cas de polyarthrite inflammatoire probable ont été relevés. Les squelettes de deux sujets âgés (tombes No 182 et 191) présentent des lésions érosives polyarticulaires bilatérales (p.ex. au niveau des articulations sacro-iliaques, sterno-claviculaires, inter-apophysaires thoraciques et lombaires, métatarso-phalangiennes et des articulations des mains). Il s'agit de l'association d'atteinte rachidienne inflammatoire non-productive et périphérique destructrice et seulement ankylosante dans un de ces deux cas (tombe No 182). L'impossibilité d'intégrer dans une entité nosologique rhumatismale, les deux cas présentés nous conduit à garder un diagnostic moins précis : celui d'une arthrite polyarticulaire inflammatoire, avec atteinte rachidienne non-productive.

Mots clés: Paléopathologie, arthropathies inflammatoires, Conquête Hongroise.

Introduction

Des destructions plus ou moins importantes des faces articulaires des os peuvent être concentrés à une inflammation chronique des articulations. L'inflammation est avant tout un mécanisme de défense visant à neutraliser l'agent agressif et à l'éliminer avec les tissus lésés. Ses effets, dépassant leur but, sont parfois néfastes. Ils agissent eux-mêmes comme un agent d'agression qui finit par altérer les fonctions articulaires (GÖMÖR et BÁLINT, 1989). Les plus fréquents des agents agresseurs responsables de l'inflammation articulaire sont les micro-organismes (arthrites et spondylites infectieuses); les micro-cristaux (goutte et chondro-calcinose); les complexes antigène-anticorps (pathologie immunologique) et les produits de la réaction immunitaire (SIMON et al., 1989).

La classification rhumatologique distingue des groupes séparés selon l'étiologie des arthropathies inflammatoires (par exemple arthrites infectieuses, rhumatismales,

métaboliques) ou selon la topographie des lésions (monoarthrite, polyarthrite, spondylarthropathies, etc.) (RYCKEWAERT, 1980).

Le diagnostic des maladies rhumatismales chez le vivant est basé sur une série de symptômes cliniques, sérologiques, immunologiques et radiologiques. En paléopathologie on ne peut observer que les séquelles osseuses morphologiques de l'inflammation; l'interprétation correcte des lésions est donc très difficile et parfois impossible, sauf certains cas évolués où les lésions sont vraiment caractéristiques. Nous connaissons dans la littérature paléopathologique plusieurs descriptions de la spondylarthrite ankylosante (KRAMAR, 1980; GOMEZ BELLARD et SANCHEZ SANCHEZ, 1989), de la goutte (ROGERS, 1984) ou de la polyarthrite érosive (KILGORE, 1989; ROTHSCHILD et WOODS, 1990).

Matériel et méthodes

Les fouilles des 269 tombes de la nécropole de Sárrétudvari-Hízóföld ont été réalisées par le Musée Déri de Debrecen (Hongrie) sous la direction de I. M.NEPPER de 1983 à 1985. Sept tombes peuvent être datées de l'Age du Bronze et 262 tombes datent des premiers trois quarts du Xème siècle (M.NEPPER, 1991).

Le sujet de notre examen est la série anthropologique provenant du cimetière du Xème siècle (les squelettes se trouvent actuellement dans les collections du Département d'Anthropologie de l'Université József Attila de Szeged). L'analyse paléoanthropologique et paléopathologique des restes osseux de 263 individus a été faite. Le sexe et l'âge au décès des squelettes ont été déterminés suivant les méthodes classiques de l'anthropologie physique (Workshop of European Anthropologists, 1980; FEREMBACH et al., 1986; MARTIN et KNUSSMANN, 1988). Chez les enfants et adolescents, d'autres méthodes (SCHINZ et al., 1952; STLOUKAL et HANAKOVÀ, 1978) ont été également prises en considération. L'âge des foetus a été déterminé à partir des formules de KÓSA (1989).

L'analyse paléodémographique préliminaire réalisée par S. OLÁH (1991) a été modifiée à cause de la découverte des restes foetaux et les modifications au niveau du nombre des tombes (M. NEPPER, 1991). Le nombre total des squelettes est ainsi 263; on peut déterminer 3 squelettes foetaux, 98 subadultes (enfants et juvéniles) et 162 squelettes adultes.

Au cours de l'analyse paléopathologique, nous avons établi le diagnostic différentiel à l'aide des examens macro-morphologiques et radiologiques.

Description

Deux cas de polyarthropathies ont été relevés dans la série de Sárrétudvari. Le squelette d'un sujet masculin âgé (tombe No 182) présente, outre une double fracture consolidée de l'avant-bras, compliquée d'une arthrose post-traumatique du coude, une série d'atteinte rachidienne et périphérique d'allure érosive. On observe des altérations érosives bilatérales et symétriques au niveau des plateaux vertébraux cervicaux (vertèbres C3 à C6); des articulations inter-apophysaires cervicales (vertèbres C2 à C4); des articulations costo-vertébrales et inter-apophysaires postérieures dorsales (vertèbres T1 à T11). Les articulations sacro-iliaques sont touchées par un processus érosif bilatéral (Fig. 1). Les mêmes signes s'observent au niveau des articulations acromio-claviculaires et sterno-claviculaires, des trochlées des talus. Des lésions érosives touchent plusieurs articulations périphériques: les articulations cuboïdo-

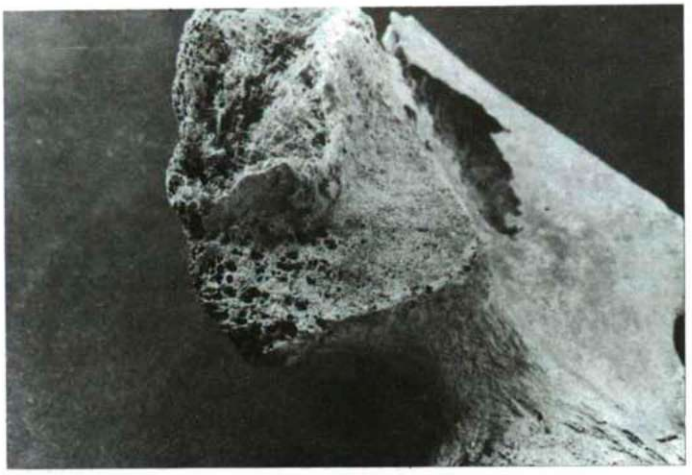

Fig. 1: Signes d'un processus inflammatoire érosif au niveau de l'articulation sacro-iliaque. L'altération est bilatérale et symétrique. (Tombe No 182, sujet adulte âgé masculin)

métatarsiennes IV. et V., les articulations IP I. et métatarso-phalangienne I. au niveau du pied gauche; l'articulation métatarso-phalangienne I. droite; les articulations de la main: os scaphoïde, grand os et os crochu, articulation métacarpo-phalangienne III., articulations IPP II., III., IV. et V. (droite) et les articulations IPP II., III. et IV.(gauche). Il n'y a pratiquement pas de signe de production osseuse (ostéophyte ou syndesmophyte).

Le processus a abouti à une ankylose au niveau de trois localisations différentes (Fig. 2). Il s'agit de la fusion des corps et des articulations inter-apophysaires postérieures des vertèbres C3 et C4, d'une ankylose postérieure des vertèbres T4 et T5 et de celle des phalanges de l'articulation inter-phalangienne proximale (IPP) V. droite. Les ankyloses, pareillement aux lésions purement érosives, ne s'accompagnent pas de production osseuse considérable. La figure 3a montre les pièces osseuses examinées. Les localisations des lésions mentionnées sont présentées dans la figure 3b.

Malgré l'absence de quelques éléments du squelette, notamment au niveau de la main et du pied gauche, on peut constater la bilatéralité des lésions.

Le deuxième cas provient de la tombe No 191 de la nécropole de Sárrétudvari. La morphologie et la topographie des lésions observées sur les restes osseux d'un sujet féminin âgé sont très similaires aux signes érosifs mentionnées dans le cas précédent; les formes ankylosantes manquant chez ce dernier. Il s'agit des lésions érosives bilatérales au niveau des articulations sacro-iliaques, sterno-claviculaires et acromio-claviculaires; des articulations inter-apophysaires thoraciques et lombaires, des régions péri-articulaires des têtes humérales; des articulations métatarso-phalangiennes des pieds et des articulations des mains: semilunaires, grand os,

articulations métacarpo-phalangiennes et interphalangiennes proximales. Il n'y a pas de signe arthrosique ou hyperostosique.

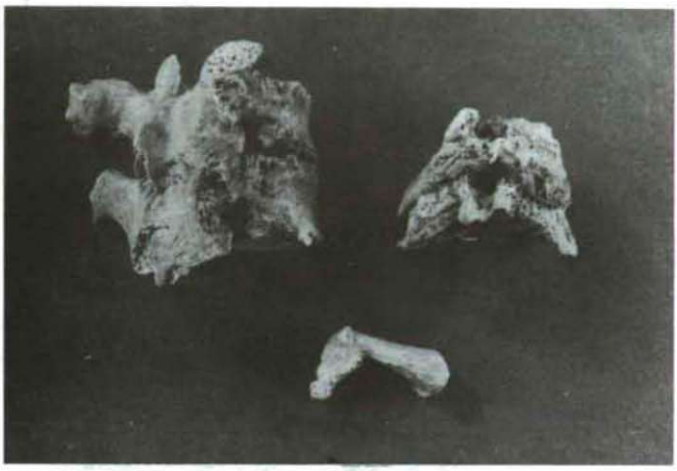

Fig. 2: Signes d'une polyarthrite aboutissant à une ankylose au niveau de trois localisations différentes: ankylose postérieure des vertèbres T4 et T5 (1), fusion des corps et des articulations inter-apophysaires postérieures des vertèbres C3 et C4 (2) et de celle des phalanges au niveau de l'articulation inter-phalangienne proximale (IPP) V. droite (3). (Tombe No 182, sujet adulte âgé masculin).

Discussion

Etant donné le bon état de conservation des squelettes, il est facile de reconnaître l'origine *ante-mortem* des lésions (dans le cas des ankyloses en particulier). La porosité sous forme micro-géodique des plateaux vertébraux cervicaux et des apophyses postérieures, ne peut pas être clairement distinguée de l'image vue dans les cas arthrosiques, mais l'absence d'ostéophytose et les manifestations périphériques à type de lésions érosives nous suggèrent plutôt un processus inflammatoire généralisé. Dans certaines localisations (carpe, doigts) l'image morphologique des érosions rappelle celle que l'on voit dans les cas actuels de polyarthrite rhumatoïde (SIMON et al., 1989), ou celle relevée sur les matériaux osseux archéologiques attribuée à cette maladie (ROTHSCHILD et WOODS, 1990; ROTHSCHILD et al., 1990). Mais la polyarthrite rhumatoïde, en général, n'intéresse pas les articulations sacro-iliaques et le rachis dorsal. L'ankylose osseuse n'est pas exceptionnelle, et touche électivement le carpe (SIMON et al., 1989). L'association des lésions périphériques, érosives ou ankylosantes, et l'atteinte des articulations sacro-iliaques est fréquente dans les spondylarthropathies (FELLMANN, 1985; ROTHSCHILD et WOODS, 1991; BARDIN, 1993; DOUGADOS, 1993). Les lésions vertébrales observées ne sont pas spécifiques, et il n'y a en particulier

aucun signe caractéristique de la spondylarthrite ankylosante (syndesmophyte). Au cours des manifestations articulaires liées au psoriasis il y a souvent coexistence d'images constructives et destructives des doigts et des orteils et ce sont typiquement les articulations interphalangiennes distales qui sont touchées (ROGERS et al., 1987; SIMON et al., 1989). L'atteinte sterno-costo-claviculaire et celles des sacro-iliaques sont fréquentes dans le syndrome acné-pustulose hyperostose ostéite (SAPHO), mais ce sont les formes hyperostosantes, en particulier vertébrales, qui prédominent (BENHAMOU, 1993).

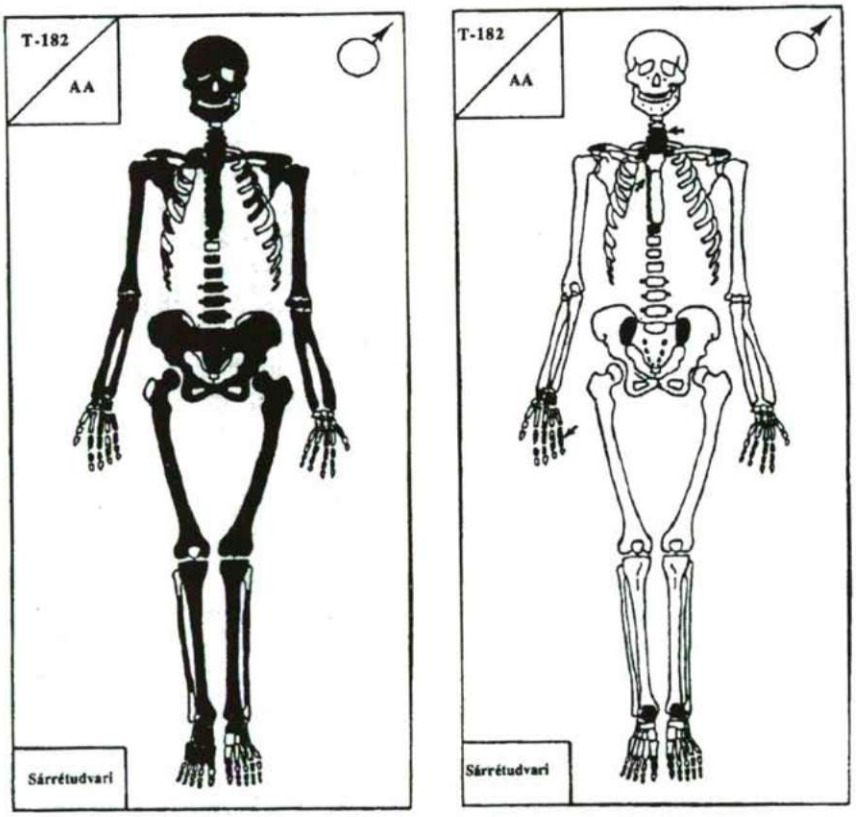

Fig. 3a: Schéma de conservation du squelette provenant de la tombe No 182 de Sárrétudvari. (noir = pièces osseuses conservées)

Fig. 3b: Localisation des lésions érosives (noir). On note le caractère bilatéral et symétrique des atteintes. (flèches: ankyloses)

Conclusions

On ne peut donc pas intégrer ces deux cas dans un cadre nosologique précis. Il s'agit de l'association d'atteinte rachidienne inflammatoire non-productive et d'atteinte périphérique destructrice et seulement ankylosante dans un de ces deux cas (tombe No 182). DONALD ORTNER, présentant dans un de ses ouvrages des témoignages d'arthrite polyarticulaire inflammatoire (API) en Amérique du Nord, indique un cas où la diversification des états pathologiques du squelette pose des problèmes pour le diagnostic différentiel, mais chacune des lésions peut être spécifiquement reliée à certains syndromes de l'API (ORTNER, 1988). L'impossibilité d'intégrer dans une entité nosologique rhumatismale les deux cas présentés nous conduit à garder le diagnostic d'une arthrite polyarticulaire inflammatoire, avec atteinte rachidienne non-productive.

Les exemples paléopathologiques d'arthropathies inflammatoires sont assez rares dans les matériaux hongrois. Deux cas de spondylarthropathie ont été relevés au cours de l'étude paléopathologique de deux séries Avars (VIIe-VIIIe siècles ap. J.-C.), représentant un ensemble de 752 squelettes. Dans un de ces deux cas, l'ankylose de plusieurs vertèbres lombaires par syndesmophytes et la présence de manifestations périphériques érosives et prolifératives, aboutissant même à l'ankylose de plusieurs articulations tarso-métatarsiennes nous suggèrent une spondylarthrite associée à des arthropathies périphériques dues à un psoriasis probable (PÁLFI, 1990). Un cas de spondylarthrite ankylosante évoluée, relevée dans une série hongroise médiévale (XIe-XIIIe siècle ap. J.-C.) a été recensé récemment par M. FERENCZ (FERENCZ, 1991).

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ÉTUDE OSTÉOARCHÉOLOGIQUE DE LA SÉRIE DE PIGNANS (VAR, FRANCE, VE-VIE SIÈCLES AP. J.-C.)

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Résumé

La fouille de sauvetage d'une nécropole gallo-romaine dans la commune de Pignans (Var, France, Ve-VIe siècles ap. J.-C.) a livré en 1978 vingt-neuf sépultures. Nous avons examiné les restes osseux souvent mal conservés de 31 individus provenant de la nécropole. Malgré cette conservation médiocre, la détermination des âges et sexes a pu être effectuée dans la plupart des cas.

L'étude paléopathologique nous révèle des signes d'altérations pathologiques. La pathologie observée est plus modeste par rapport aux séries en meilleur état de conservation. A l'aide de l'examen macro-morphologique et radiologique, 9 cas pathologiques ont pu être relevés dont les plus fréquents sont les altérations d'allure dégénérative (arthrose vertébrale et extra-spinale). Une périostose généralisée - vraisemblablement d'origine infectieuse - et des pathologies des insertions musculaires sont à mentionner.

Mots clés: Paléopathologie, enthésopathies, périostose, période gallo-romaine, France.

Introduction

A la suite de la destruction d'une tombe au cours des travaux de viabilisation d'un terrain à lotir, une fouille de sauvetage a été entreprise en 1978 par le Centre Archéologique du Var dans la commune de Pignans (Saint-Roch) (Fig. 1).

Vingt-neuf inhumations et 4 réductions ont été fouillées. Deux tombes, découvertes fortuitement un peu à l'écart, suggèrent que le cimetière n'a pas été fouillé dans sa totalité (GÉBARA et PASQUALINI, sous presse). Dans le cas des tombes No. 13, 25 et 30, gravement endommagées par les travaux, les restes osseux n'ont pas été relevés. Un bol en céramique paléochrétienne estampée, déterré aux abords de la tombe No. 5 et le type des tombes permettent d'assigner au cimetière une durée assez longue au cours des Ve et VIe siècles ap. J.-C. (BONIFAY et PASQUALINI, 1978).

L'absence de mobilier dans la plupart des inhumations du cimetière (à l'exception de 2 tombes avec offrandes alimentaires) doit être attribuée à la christianisation des populations de la région de Pignans à cette époque. Une seule tombe était orienté Sud-Nord, les autres Ouest-Est. Cette dernière orientation était devenue habituelle aux Ve-

VIe siècles ap. J.-C. (BAYLEY-YOUNG, 1977; BONIFAY et PASQUALINI, 1978).

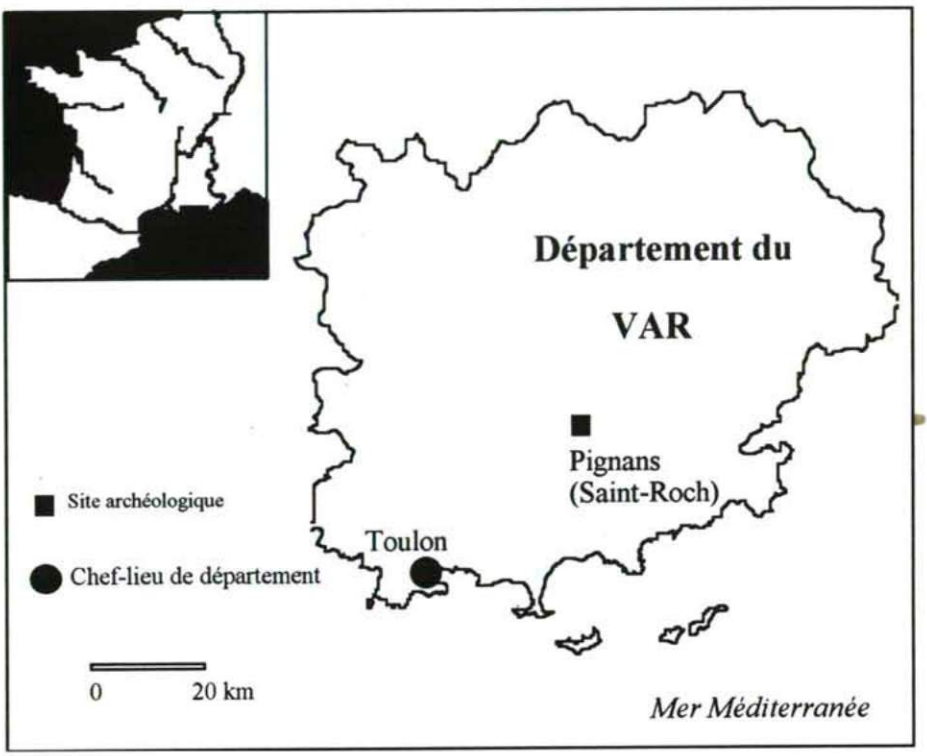

Fig. 1: Localisation géographique de la nécropole de Pignans (Saint-Roch).

La majorité des tombes étaient en pleine terre, cinq sont sous *tegulae*. Dans quatre tombes en pleine terre, des inhumations superposées ont été relevées, qui sont toujours le résultat de la réouverture de certaines tombes en vue d'une réutilisation, probablement dans un but pratique (économie de place) (COLLECTIF, 1978).

A proximité des sondages en 1977 avaient découvert les vestiges d'un établissement gallo-romain dont l'occupation est attesté du Ier au IIème siècles ap. J.-C., puis au cours de l'Antiquité tardive. Il s'agit vraisemblablement d'une *villa* dont quelques matériaux ont été réemployés aux Ve-VIe siècles dans la nécropole de Saint-Roch (BONIFAY et PASQUALINI, 1978).

Matériel et méthodes

Nous avons examiné les restes osseux de 31 individus provenant de la nécropole. Du fait de conditions météorologiques particulièrement mauvaises (pluies, sols humides), les archéologues avaient essayé de sauver les squelettes trempés et boueux à l'aide d'une résine (*Plexigum*). Cette "consolidation *in situ* " a rendu très

difficiles les travaux de restauration et l'examen anthropologique.

Les squelettes sont conservés dans les collections du Centre Archéologique du Var (Toulon).

Malgré toutes les précautions, les squelettes, souvent mal conservés au moment de leur prélèvement, ont été à nouveau endommagés lors de la restauration. La majorité des squelettes est fragmentaire et il n'y a pas de squelettes en bon état. Les squelettes en état moyen, bien qu'assez complets, ont des os fragiles, friables; les surfaces sont souvent érodées *post mortem*. Malgré cette conservation médiocre, la détermination des âges et sexes a pu être effectuée dans la plupart des cas. Le nombre total des squelettes est de 31; on peut déterminer 8 squelettes subadultes (S) et 23 adultes (A). Le sexe et l'âge au décès des squelettes ont été déterminés suivant les méthodes classiques de l'anthropologie physique (Workshop of European Anthropologists, 1980; FEREMBACH et al., 1986; MARTIN et KNUSSMANN, 1988). La répartition des sujets selon les sexes et tranches d'âge est présentée dans le Tableau 1.

L'étude paléopathologique a été réalisé à l'aide de l'examen macro-morphologique et radiologique.

Discussion

L'étude paléopathologique nous révèle des signes d'altérations pathologiques. La pathologie observée est plus modeste par rapport aux séries en meilleur état de conservation. A l'aide de l'examen macro-morphologique et radiologique, 9 cas pathologiques ont pu être relevés dont les plus fréquents sont les altérations d'allure dégénérative (arthrose vertébrale et extra-spinale). Une périostose généralisée et des pathologies des insertions musculaires sont à mentionner.

Tableau 1: Répartion des sujets selon les sexes et tranches d'âge dans la série de Pignans. S : subadultes, A : adultes

	Masculin	Féminin	Indéterminé	Total
S - Inf. I	---	---	5	5
S - Inf. II	---	---	2	2
S - Juv.	1	---	---	1
A - jeune	---	1	2	3
A - mature	3	6	1	10
A - âgé	2	2	---	4
A - indéterminé	2	1	3	6
Total	8	10	13	31

La description des cas les plus simples est détaillée dans le Tableau 2; nous nous limiterons ici à la présentation du diagnostic différentiel des deux cas les plus importants.

Tombe No. 26.

Description : Sujet masculin, adulte mature, état fragmentaire de conservation.

Le caractère le plus remarquable du squelette de ce sujet masculin, outre une autre pathologie banale (carie dentaire), est l'altération décelable sur les deux humérus au niveau des insertions des muscles grand pectoral *(M. pectoralis major)* et grand rond *(M. teres major)*. Ce caractère se manifeste sous la forme de fosses longitudinales délimitées dans les parties antéro-supérieures des deux humérus. Les empreintes des mêmes insertions sont beaucoup moins marquées à gauche. La Figure 2 montre que le phénomène n'est que partiellement bilatéral : Côté droit : fosse (27x4,5x3,5 mm) à l'insertion du grand rond, mais également une fosse importante et délimitée

(23x5x4mm) à l'insertion du grand pectoral. Côté gauche : une fosse (20x3x2mm), à l'insertion du grand rond, et une faible empreinte (15x2x0,5mm) à l'insertion du grand pectoral. Il faut aussi mentionner une empreinte linéaire (15x1x2mm) située de 200 à 350 mm au dessous de la dépression sous-deltoidienne, au niveau de l'insertion du muscle brachial antérieur.

Tableau 2: Paléopathologie des altérations relevées sur 9 squelettes de la série de Pignans. Age : AM = adulte mature, AA = adulte âgé. Sexe : F = féminin. M = masculin.E = état de conservation : f = fragmentaire, m = moyen.

Tombe	Age	Sexe	E.	Paléopathologie
1	AA	F	m	arthrose temporo-mandibulaire; discarthrose dorsale
5A	AM	F	f	discarthrose dorsale
11A	AM	F	m	discarthrose et arthrose interapophysaire lombaire; syndrome de la "queue de l'astragale"
11B	AM	M	m	spondylolyse isthmique; arthrose de la charnière lombo-sacrée; coxarthrose débutante; syndrome de la "queue de l'astragale"; anomalie ou pathologie de zone d'inserion (clavicule)
17	AA	M	m	cervicarthrose; arthrose de la charnière lombo-sacrée; processus dégénératif probable de l'articulation sacro-iliaque droite
19	AM	F	f	arthrose atlo-odontoïdienne; *cribra orbitalia*
24A	AA	F	f	arthrose atlo-odontoïdienne; arthrose cervicale; enthésopathies
26	AM	M	f	hypersollicitation de zone d'insertions (humérus)
29	AM	M	m	fractures consolidées (clavicule, humérus) ostéite (périostose généralisée d'origine infectieuse probable); coxarthrose débutante; arthrose (poignet, main); arthrose atlo-odontoïdienne, arthrose interapophysaire cervicale et lombaire

Interprétation : L'insertion du grand pectoral et du grand rond sous forme de fosse longitudinale, bien délimitée, est connue comme une variation anatomique dans la littérature anthropologique (SAUNDERS, 1978). Le pourcentage élevé de ce caractère dans une population des Ve-VIe siècles avec une prépondérance chez les hommes a été décrit récemment par CASTEX (1990). La particularité de la tombe No 26 réside surtout dans le profondeur de la fosse et dans son inégalité latérale. L'étiologie de ces lésions est encore incertaine. Bien que SAUNDERS (1978) suggère une interaction entre contraintes biomécaniques et modalités particulières de croissance dans la formation de cette anomalie, la littérature des lésions pathologiques liées aux activiés physiques (MERBS, 1983, KENNEDY, 1989) ne tient pas compte de l'importance de ce phénomène. Les nouveaux résultats de la physiopathologie des enthésopathies d'insertion tendineuses nous démontrent cependant que l'action tendino-musculaire peut provoquer des lésions intra-osseuses profondes. L'aspect de ces fosses observées

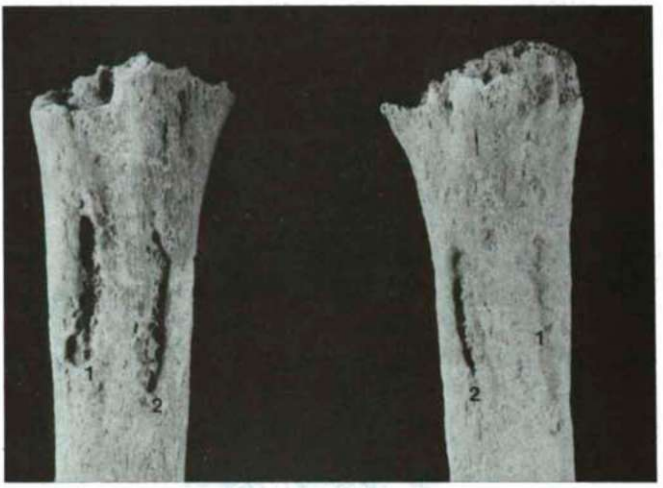

Fig. 2: Insertion des muscles grand pectoral (1) et grand rond (2) sous la forme de fosses sur les deux humérus. On note la prédominance de droite des altérations. (Pignans; tombe No 26, sujet adulte mature masculin)

sur l'humérus droit en particulier, dans cette hypothèse suggère le diagnostic de géodes osseuses dystrophiques poly-microtraumatiques par arrachement, observées en clinique dans d'autres localisations (HUSSON et al., 1991).

L'association avec l'altération de l'insertion du muscle brachial antérieur et, d'après la littérature, le lien étroit des fosses humérales avec l'âge, notamment leur haute fréquence au cours de l'adolescence (SAUNDERS, 1978) nous permettent de penser que ces lésions se produisent sous l'effet d'un surmenage musculaire pendant la phase active de la croissance.

Tombe No. 29.

Description : Sujet masculin, adulte mature, état moyen de conservation.

Le caractère le plus remarquable est la présence d'appositions généralisées sur les os. Dans ce cas, l'enlèvement des couches constituées de résine et d'argile, collées très fortement aux pièces osseuses a été particulièrement délicat. Quelques valeurs métriques prises sur le squelette de ce sujet masculin de taille moyenne (170,31±3,4cm) indiquent déjà des processus pathologiques. Les valeurs anormalement élevées des indices de robustesse (par exemple indice de robustesse au périmètre du fémur gauche : 25,87; indice de robustesse de l'ulna gauche : 20,85) sont dues aux appositions ostéopériostées. Les altérations pathologiques détectées sur le squelette que nous examinons ici, sont présentées dans le Tableau 2.

Interprétation : Les altérations arthrosiques souvent très évoluées sont des conséquences possibles de processus vraisemblablement dégénératifs, dans le cas des arthroses interapophysaires postérieures unilatérales, des arthroses radio-cubitales inférieures et de la rhizathrose du pouce en particulier.

Des lésions d'origine traumatique plus intéressantes se manifestent en plusieurs localisations. Une fracture consolidée de la clavicule droite peut être relevée. La fracture (ou une infection) a provoqué la production d'exostoses importantes au niveau de la face inférieure de la clavicule, au bord de la gouttière du sous-clavier, délimités par le tubercule conoïde et le trou nourricier. Dimensions : 22x6x8 mm et 35x9x17 mm. Les exostoses correspondent probablement à l'ossification de l'insertion de l'aponévrose clavi-coraco-axilliaire (plus exactement la gaine du sous-clavier) aux lèvres de la gouttière. Dans les fractures de la clavicule, ce muscle joue un rôle important, car c'est lui qui produit le mouvement de bascule en bas du fragment claviculaire externe (PATURET, 1951). Une exostose importante de dimensions 34x11x15 mm, est visible sur l'humérus droit, juste au dessus de la dépression sous-deltoïde, en face du trou nourricier. Elle correspond vraisemblablement à l'insertion du muscle deltoïdien. Une fracture diaphysaire consolidée de l'humérus droit est probable.

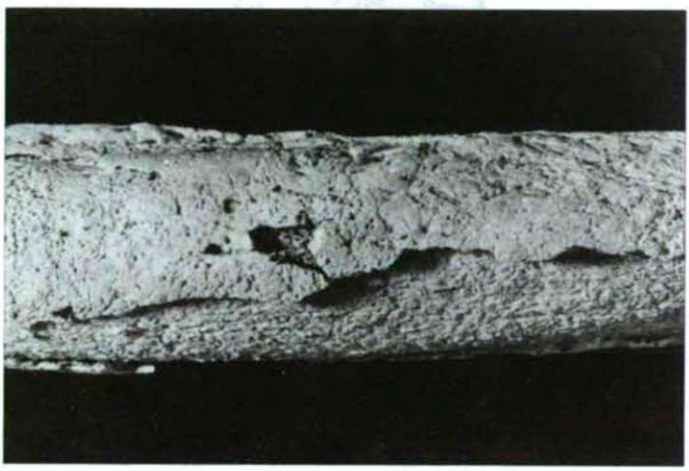

Fig. 3: Périostose évoluée au niveau du fémur gauche. La couche de la néoformation osseuse est séparée de l'os compact. (Pignans; tombe No 29, sujet adulte mature masculin)

Le caractère le plus remarquable est la présence des appositions périostées sur les os. Une périostose généralisée, symétrique et bilatérale est visible sur le squelette. Une apposition périostée étendue devait recouvrir presque entièrement les os longs des membres inférieurs : les fémurs, les tibias et les péronés. Du fait de la fragmentation des os, un examen macroscopique révèle d'une part l'intégrité de la corticale sous-jacente et d'autre part que la périostose est séparée de l'os compact (Fig. 3). L'analyse radiologique confirme cette observation (Fig. 4). L'épaisseur des appositions varie de 2 et 6 mm, plus épaisse et irrégulière dans la région des insertions musculaires. La surface extérieure des os coxaux, les surfaces antérieures des vertèbres lombaires sont également atteintes. Les deux humérus sont particulièrement touchés, surtout les

parties distales (Fig. 5). Les os des avant-bras sont déformés ("gonflés") et couverts d'appositions périostées.

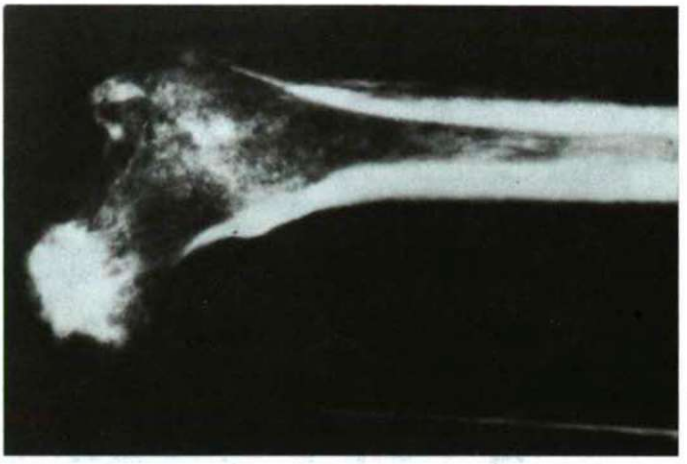

Fig. 4: Cliché radiologique relevant des appositions périostées à la partie supérieure du fémur gauche.(Cliché J. Bérato). (Pignans; tombe No 29, sujet adulte mature masculin)

Fig. 5: Signes d'une ostéite évoluée au niveau de la partie distale des humérus. (Pignans; tombe No 29, sujet adulte mature masculin)

L'interprétation des cas d'atteinte de périostite est un domaine problématique de la paléopathologie. L'hyperossification périostée est une des modalités réactionnelles de l'os à une agression traumatique, microbienne ou autre (MAFART, 1979; DASTUGUE et GERVAIS, 1992). Une périostite tibiale localisée pourrait évoquer un syndrome de

fatigue (BRODY, 1981), mais le caractère généralisé des lésions est évocateur d'une infection disséminée (ORTNER et PUTSCHAR, 1985). Bien que plusieurs conditions pathologiques puissent être associées avec une réaction ostéopériostée, on pourrait mentionner que la morphologie et la disposition des lésions sur le squelette pourraient se rapporter à une tréponématose (HACKETT, 1976, STIRLAND, 1991). Malheureusement l'état fragmentaire du squelette en général et particulièrement celui des tibias nous empêchent d'affirmer ce diagnostic, qui repose sur une sémiologie osseuse assez peu spécifique chez l'adulte, mais qui vient d'être défini dans la région à cette période sur des restes foetaux (DUTOUR et al., 1991b; PÁLFI et al., 1992b).

Il est évident dans ce cas qu'une réaction locale après une infection doit être exclue, étant donné le caractère généralisé des lésions. L'absence de réaction endostale permet d'exclure la fluorose et de l'hyperostose généralisée de Van Buchem (BUFFARD et al., 1976; ORTNER et PUTSCHAR, 1985). L'hypervitaminose A, signalée par ailleurs chez l'*Homo erectus* (WALKER et al., 1982) peut être écartée étant donné que, dans l'hypervitaminose A, l'hyperostose de la diaphyse moyenne est essentiellement sous-périostale et non supra-périostale comme dans le cas étudié ici. L'ostéo-arthropathie hypertrophiante de Pierre-Marie, dont un cas probable provenant de la même période a été recensé par MAFART (1979) (nécropole de l'abbaye Saint-Victor de Marseille, IVe-VIe siècles ap. J.-C.), est également caractérisée par une prolifération périostée bilatérale et symétrique. Mais cette maladie est toujours exclusivement diaphysaire, les os les plus touchés sont le radius, l'ulna, le tibia et le fibula; l'humérus et le fémur sont plus rarement impliqués et les os plats sont respectés (SIMON et al., 1989). Dans notre cas, c'est la localisation des lésions qui nous permet d'exclure cette hypothèse.

Ainsi, le diagnostic le plus plausible est celui d'une ostéopériostite vraisemblablement d'origine infectieuse. Les altérations au niveau de la métaphyse distale de l'humérus droit peuvent évoquer une périostite compliquée d'une ostéomyélite chronique (BRAUNER et al., 1982), ressemblant au cas médiéval décrit par PROMINSKA (1984). Bien que l'aspect des coulées périostées des tibias et les déformations pathologiques des ulnas puissent révéler une atteinte syphilitique, comme dans le cas présenté par STIRLAND (1991), les autres localisations et la coincidence possible avec des lésions d'origine traumatique nous suggèrent plutôt une ostéïte généralisée suite à une infection pyogénique disséminée probable, sans aucune précision sur le germe en cause.

Conclusions

Malgré la conservation médiocre des squelettes, l'étude paléopathologique des squelettes nous révèle des signes d'altérations pathologiques chez 9 sujets. La pathologie observée est plus modeste par rapport aux séries en meilleur état de conservation et provenant de cette époque historique, notons par exemple les séries de la Porte d'Orée (DUTOUR et al., 1991a), Solliès-Toucas (PÁLFI et al., 1992a) ou Costebelle (PÁLFI et al., 1993).

A l'aide de l'examen macro-morphologique et radiologique, 9 cas pathologiques ont pu être relevés dont les plus fréquents sont les altérations d'allure dégénérative (arthrose vertébrale et extra-spinale). Notons le taux élevé de l'arthrose vertébrale dans la population adulte (8 cas/23 sujets adult; 34,8 %). Outre des localisations plus communes (cervicarthrose, arthrose lombaire ou de la charnière lombo-sacrée), l'étude a relevé plusieurs cas d'arthrose atlo-odontoïdienne (3cas/23 adultes, 13 %) (Tableau 2).

La fréquence élevée de l'arthrose atlo-odontoïdienne dans une série antique varoise a été déjà rapportée par BÉRATO et DUTOUR (Bérato et DUTOUR, 1989; BÉRATO et al., 1990). Ils ont relevé 19 atteintes de cette articulation dans un ensemble de 163 squelettes adultes provenant de la nécropole du Haut-Empire de Saint-Lambert. Les auteurs étaient frappés par ce phénomène car, à cause du taux élevé des incinérations, ce n'était que 45 pièces osseuses de cette localisation qui étaient présentes à l'examen. La fréquence de l'arthrose atlo-odontoïdienne est donc de 42 % dans cet ensemble. Etant donné que l'état de conservation des squelettes est souvent fragmentaire dans la série de Pignans, la fréquence "réelle" de cette affection devait être plus élevée également dans cette population.

L'importance des cas discutés plus haut : la périostose généralisée d'origine infectieuse (Tombe No. 29) et la pathologie des insertions musculaires (Tombe No 26), réside dans leur intérêt pour le diagnostic différentiel des lésions pathologiques observées sur des squelettes humains anciens.

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PALEOPATHOLOGICAL DIAGNOSIS AND INTERPRETATION OF SERONEGATIVE SPONDYLARTHROPATHIES FROM THE 17TH CENTURY

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Abstract

This paper discusses two possible cases of seronegative spondylarthropathy. The bony remains of two adult male skeletons from a 17th century cemetery at Bácsalmás exhibit erosive and proliferative pathological lesions. The osteoarticular alterations affect the skeletons in numerous locations. The major pathology in both cases is fusion of the lumbar vertebrae by the formation of syndesmophytes. Some thoracic and cervical segments are also affected. Inflammatory changes, in the form of erosive articular (sacroiliac and acromioclavicular joints) or periarticular (humeri and calcanei) lesions, and entesopathies of some ligament or tendon insertions can be detected in both cases. The morphology of the lesions emphasizes ankylosing spondylitis as a form of undifferentiated seronegative spondylarthropathy. In the knowledge of the heritability of the factors which predispose to the disease, and the neighboring location of the two graves in the cemetery, a family relationship may be suggested between the two individuals.

Key words: paleopathology, 17th century, seronegative spondylarthropathies, ankylosing spondylitis.

Introduction

Pathological changes involving the joints are frequently seen in paleopathological material. In addition to the much more common degenerative joint diseases, different types of inflammatory joint disorders can be recognized (ORTNER and PUTSCHAR, 1985).

The group of seronegative spondylarthropathies (SNSA) is an entity distinct from rheumatoid arthritis (RA). Serologically, a common feature of the group is the absence of the rheumatoid factor (an anti-immunoglobulin) from the serum (ROGERS and WALDRON, 1986). Another common serological character is the high association with the histocompability antigen HLA-B27 (GÖMÖR and BÁLINT, 1989).

As for the skeletal manifestations, spondylarthropathy represents a subset of arthritis, characterized by erosive joint disease, ossification sites of tendons and

ligaments, and a tendency to spine and sacroiliac fusion. This group of arthropathies includes ankylosing spondylitis (AS), Reiter's syndrome, psoriatic arthritis, entheropathic arthropathies and undifferentiated spondylarthropathies (RESNICK and NIWAYAMA, 1988).

The medical literature and studies of series of documented defleshed bones have provided data for the paleopathological identification of spondylarthropathies (e.g. ORTNER and PUTSCHAR, 1985; RogeRs et al., 1987; ROTHSCHILD and WOODS, 1991). AS, characterized by symmetric sacroiliitis, spinal new-bone formation and some typical extraspinal changes, is the best known of the group and has been most frequently diagnosed in archeological specimens. Osteoarcheological collections document the existence of the disease long before the modern era (e.g. STEINBOCK, 1976; KRAMAR, 1980, 1987; ORTNER and PUTSCHAR, 1985; GOMEZ BELLARD and SANCHEZ SANCHEZ, 1989; PÁLFI, 1990; FERENCZ, 1991). We also have data concerning the existence of other forms of spondylarthropathies from prehistorical periods, for example psoriatic arthritis from an Early Holocene African population (DUTOUR et al., 1994).

Materials and Methods

A paleopathological examination was carried out on 83 skeletons from a 17th century cemetery at Bácsalmás-Homokbánya. The cemetery consisted of 91 graves and was excavated in 1993 by the archeologist of the Thorma János Museum at Kiskunhalas. The coins found in some graves revealed that the cemetery was in use during the 17th century (WICKER, manuscript). Historical data helped with identification of the excavated cemetery (IVÁNYI, 1909; BOROVSZKY, 1910). It was used by a Serbian community living at Bácsalmás during the Turkish occupation of Hungary.

The examined skeletons are to be found in the collection of the Department of Anthropology at József Attila University. The majority of the skeletal material is in a good or medium state of preservation.

The sex and age at death were determined with traditional methods used in historical anthropology (FEREMBACH et al., 1979; KNUSSMAN, 1988). The differential diagnosis was based on macromorphological and X-ray methods. Both clinical and paleopathological special literature were used (e.g. ORTNER and PUTSCHAR, 1985; RESNICK and NIWAYAMA, 1988; LE LOET and BRUNO, 1990; BAHK, 1994). Radiological analyses were carried out at the Department of Radiology, Szeged Hospital.

Results and Discussion

Signs of polyarticular inflammatory processes with proliferative spinal affection were detected in two cases in the skeletal material from the Bácsalmás cemetery. The two cases are presented separately; however, the similarity of the lesions demands a common discussion.

Descriptions:

Case No. 1: Grave No. 80. Male skeleton. Aged adult; good state of preservation.

The anthropological examination revealed that the adult man was at last 70 years old when he died. Besides the typical age-related skeletal changes, the *in vivo* lack of the complete set of teeth, the severe atrophy of the alveolar processes and the typical symmetrical thinness of the parietal bones (identical to that in the case presented by PERROT and BILLARD (1982) in the paleopathological literature) are to be mentioned.

In spite of the advanced age of this individual at death, the only sign of degenerative articular process is the slight secondary osteoarthrosis of the left ankle, following a healed fracture of the left medial malleolus.

Bilateral enthesopathic aspects, in the form of erosions and slight new-bone formation at the site of tendon insertions, can be observed on the posterior part of the calcanei and the ischial tuberosities.

Signs of a more advanced stage and bilateral erosive process are to be seen in both sacroiliac joints (Fig. 1). Multiple erosions and remodeled holes are present on the auricular surfaces of the ilia and the sacrum; the process predominates in the ilium.

The major pathological changes of the skeleton are the spinal lesions. As all of the elements of the spine are available, the location of the alterations can be easily recognized.

- Cervical spine: C1-C2: slight marginal osteophyte formation in the median atlanto-axial joint. C3-C7-(T1): erosive changes of the vertebral bodies and some of the apophyseal joints. Advanced stage alterations in segments C5-C6, which display fusion by marginal osseous bridging and ankylosis in the discovertebral and apophyseal joints (Fig. 2).
- -Thoracic spine: T1-T7: no pathological changes. T7-T12: localized, erosive discovertebral lesions. Signs of inflammatory processes of the costovertebral joints in segments T9- T12. Collapse of the anterior margin of T11 and bony ankylosis of T11-T12 (Fig. 3a).
- -Lumbar-spine: L1-L5: the five lumbar segments are fused in a single block. (Fig. 3b-d). Syndesmophyte-like new-bone formation, with preservation of disc space, is present in segments L2-L3 and L3- L4. Extensive central and peripheral discovertebral lesions are also present in segments L1-L2 and L4-L5 (Fig. 3c).

Case No. 2: Grave No. 90. Male skeleton. Aged adult; very good state of preservation.

The age at death of the second subject must have been considerably lower than in the first case (in case No. 2, the man died in his sixties).

The quasi-complete and robust skeleton presents a relatively good state of

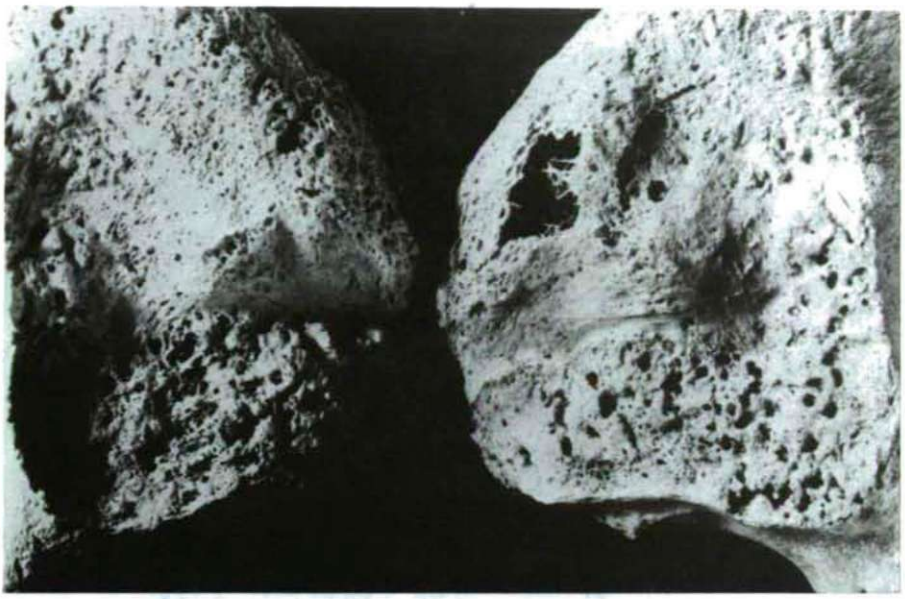

Fig. 1: Erosive changes on the two ilia, suggesting bilateral symmetric sacroiliitis. (Case No. 1: Grave No. 80)

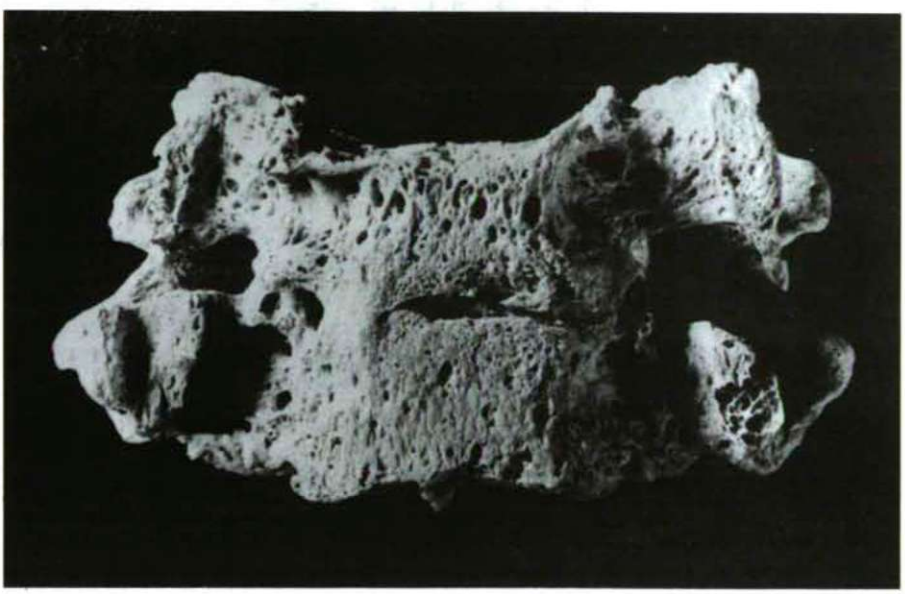

Fig. 2: Ankylosis of vertebrae C5-C6 by marginal osseous bridging. (Case No. 1: Grave No. 80)

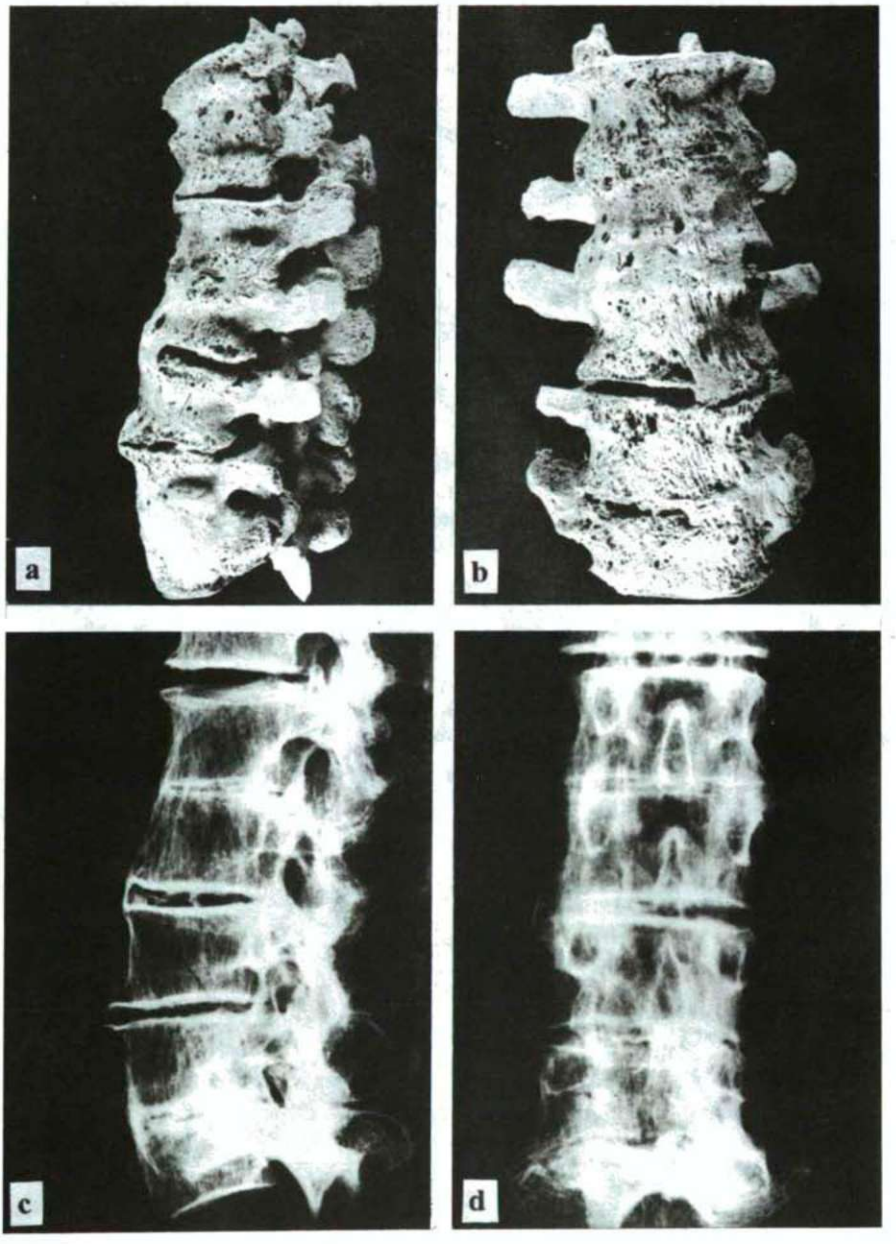

Fig. 3a-d: Lateral and antero-posterior macroscopic (a-b) and X-ray pictures (c-d) of the lumbar and thoraco-lumbar segments. Syndesmophytosis and ankylosis are to be mentioned. (Case No. 1: Grave No. 80)

mineralization. As for the pathological conditions, a lytic bony response to the abscessed second incisor, canines and second premolar can be observed on the left maxilla. The significant alveolar resorption is indicative of periodontal disease. Lesions due to a previous trauma are seen on the skeletal remains of the left hand. The second and third left metacarpals and the second proximal phalanx exhibit signs of healed fractures, deformation and posttraumatic arthrosis.

Enthesopathic alterations can be observed in several locations. Bilateral abnormalities of the ligament attachments (proliferations and erosions) are detected on the ischial tuberosities, the trochanters of the femurs, the posterior and inferior parts of the calcanei (Fig. 4) and the radial tuberosities.

Bilateral articular and periarticular erosive lesions affect both shoulders (greater tubercles of the humeri and acromioclavicular joint surfaces of the clavicles and scapulae) and wrists. Only a more precise morphological examination can reveal the very slight, early stage alterations of the two side sacroiliac joints.

The most important pathological alterations affected the vertebral column.

-Cervical spine: C1-C2: periarticular osteophytes in the median atlanto-axial joint (atlas), with partial calcification of the apical ligament to the dens (axis). C3-C7: erosive changes and marginal new-bone formation affect the vertebral bodies and apophyseal joints. Segments C4-C6 are most strongly involved (Fig. 5).

-Thoracic spine: No pathological signs between segments T1-T4. T4-T8: erosive lesions in the apophyseal joints. T6-T12; slight, bilateral erosions in the costovertebral joints. The signs of Schmorl's nodes must be mentioned.

-Lumbar spine: L1-L5: ankylosis of the five lumbar segments (Fig. 6a-d). Widespread marginal bony bridges lead to fusion of the vertebral bodies. The intervertebral discs are partially ossified, especially between L1 and L3 (Fig. 6c-d).

Discussion

The considerable similarity of the morphology and skeletal patterns of the detected lesions in the two cases allow their discussion together.

There are some pathological lesions in both cases, where the morphological character suggest localized independent processes, such as the healed fractures and secondary posttraumatic arthroses (cases 1 and 2), or the dental and periodontal disease in case 2.

The widespread articular and periarticular lesions suggest the possibility of generalized diseases in both cases.

The morphology of the erosive lesions observed in some extraspinal and vertebral locations suggests inflammatory joint (or periarticular) disease (ROTHSCHILD et al., 1990). The affection of the sacroiliac joints, especially in case 1, reveals bilateral sacroiliitis. The alterations predominate in the ilium in both cases. (According to the

Fig. 4: Enthesopathies on the posterior and inferior parts of the calcanei. (Case No. 2: Grave No. 90)

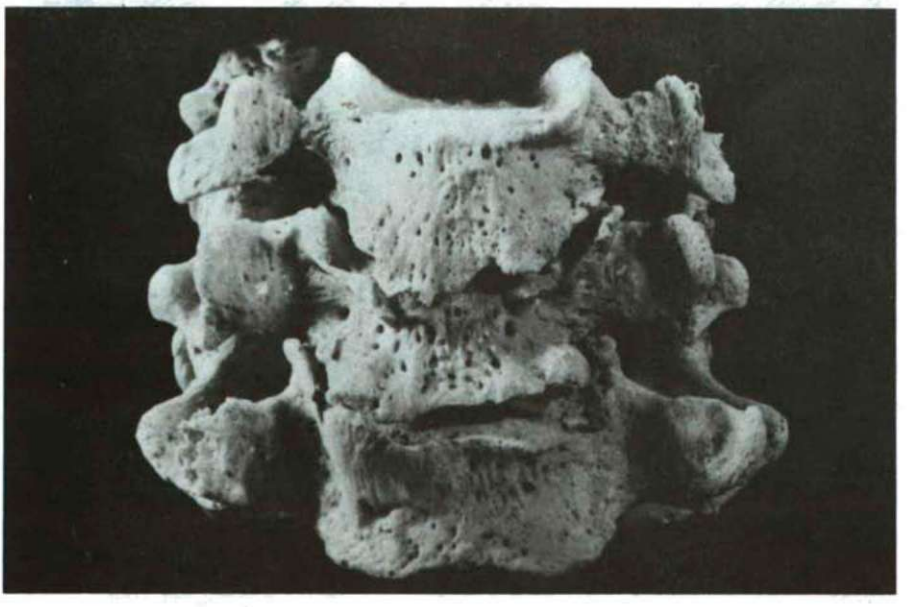

Fig. 5: Marginal proliferative lesions on vertebrae C4-C6. (Case No. 2: Grave No. 90)

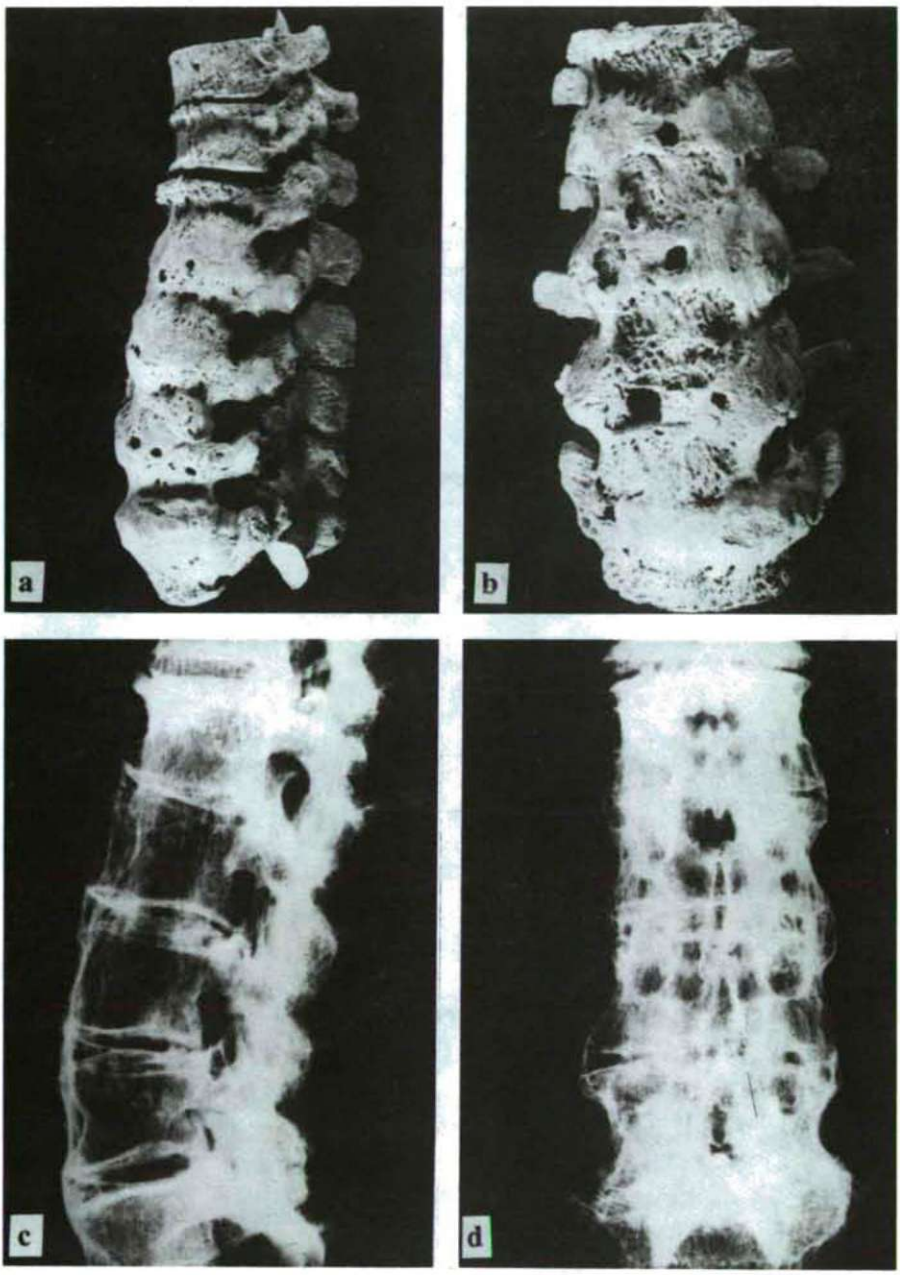

Fig. 6a-d: Lateral and antero-posterior macroscopic (a-b) and X-ray pictures (c-d) of t\he lumbar and thoraco-lumbar segments. Syndesmophytosis and ankylosis are must be mentioned. (Case No. 2: Grave No. 90)

literature data (RESNICK and NIWAYAMA, 1988), this phenomenon results from the iliac cartilage being thinner than that of the sacrum.)

The erosive lesions localized to the central subchondral portions of the discovertebral junction are associated with reactive sclerosis. There are no signs, however, of irregular single or multifocal osteolytic lesions, geode-like erosions, irregular destruction of the vertebral bodies, sequestrum formation or abscesses, as in the case of infective spondylitis (BAHK, 1994).

The presence of the proliferative lesions and the para-erosional new-bone formation is in marked contrast to the minimal or absent para-erosional new-bone formation noted in RA (ROTHSCHILD et al., 1990; ROTHSCHILD and WOODS, 1991).

The proliferative lesions are possible consequences of a multifocal enthesopathy. (The enlarged term of enthesis is used here according to FOURNIÉ and FOURNIÉ (1992) to denote all of the sites of insertion of ligaments, tendons, capsules and intervertebral discs into the bone.) In the two examined cases, proliferative enthesopathies affect predominantly the intervertebral discs, e.g. the osseous bridging through the anulus fibrosus (syndesmophytes) on the lumbar and cervical spine, the apophyseal joints and some extravertebral ligament insertions.

The enthesopathic character of some extraspinal ligament or tendon insertions can be associated with numerous metabolic, inflammatory, infectious, traumatic or microtraumatic conditions (SIMON et al., 1991). Enthesopathies in the observed and above-mentioned locations (bicipital tuberosity of the radius, trochanters of the femurs and ischial tuberosities) have already correlated with the syndromes of overuse in the paleopathological literature (DUTOUR, 1986; MILLER, 1992; PÁLFI, 1992). In our case, however, the more widespread character of the lesions and their coexistence with the observed inflammatory changes help us to exclude this possibility.

There is an association of proliferative vertebral lesions and extraspinal enthesopathies in cases of diffuse idiopathic skeletal hyperostosis (DISH) (ARLET and MAZIERES, 1985; GÖMÖR and BÁLINT, 1989). In DISH, however, the massive, vertical osteophytes are seen predominantly on the right anterolateral surface of the vertebral bodies of the thoracic spine, and the disc spaces and facet joints are always normal (ROGERS et al., 1987).

According to the morphology and skeletal pattern of the observed erosive and proliferative inflammatory lesions and the specific literature data (ROGERS and WALDRON, 1986; RESNICK and NIWAYAMA, 1988; GÖMÖR and BÁLINT, 1989; ROTHSCHILD and WOODS, 1991; DOUGADOS, 1993; BAHK, 1994), the presumed diagnosis in the examined cases is seronegative spondylarthropathy.

Within the group of SNSA, the classification and the differential diagnosis are often difficult, especially in the early stage or in atypical cases (MAU et al., 1987; ZEIDLER, 1987). The relatively well-developed character of the observed lesions help their more precise diagnosis.

The symmetric sacroiliitis, spinal syndesmophytosis and fusion, and the above-

mentioned extraspinal enthesopathies (e.g. the changes detected on the inferior surface of the calcanei) suggest the diagnosis of AS (RYCKEWAERT, 1980; GRAN et al., 1984; SIMON et al., 1984; FELLMAN, 1985; ROGERS et al., 1987; GÖMÖR and BÁLINT, 1989; LE LOET and BRUNO, 1990).

The symmetric pattern of the sacroiliitis is an important clue in this disease and may permit its differentiation from other disorders, such as psoriasis, Reiter's syndrome, RA and infection (RESNICK and NIWAYAMA, 1988).

As for the syndesmophytosis, the connection of the vertical outgrowths to the vertebral edges allows their differentiation from the paravertebral ossification of psoriasis and Reiter's syndrome (which begins at a distance from the vertebral body and intervertebral disc) (RESNICK and NIWAYAMA, 1988; LE LOET and BRUNO, 1990). In AS, its marked tendency towards chondral-type ossification of fibrosed tissues is of particular importance. This ossification leads to "bamboo spine" deformity by widespread syndesmophytosis and the ossification of multiple intervertebral discs, which result in an undulating spinal contour (BAHK, 1994).

The abscence of specific locations which may be expected in such well-developed cases can similarly help the diagnosis. The lack of lesions of the distal interphalangeal joints does not indicate the diagnosis of a psoriatic arthritis (CASALIS, 1985; BAHK, 1994). The presence of the enthesopathies of the calcanei and the detected vertebral erosions, and the lack of sternoclavicular arthritis exclude another type of seronegative arthropathies, spondarthritis hyperostotica pustulo-psoriatica or SAPHO syndrome (DIHLMANN, 1993; GERSTER et al., 1993; NAGEL et al., 1993).

Conclusions

The paleopathological analysis and the subsequent differential diagnostic study of the osteoarticular lesions of the two adult male skeletons from graves 80 and 90 reveal that the two individuals suffered from SNSA. The literature data demonstrate that the most probable diagnosis within the group of SNSA is that of ankylosing spondylitis.

The inequality of the stage of involvement in different locations appears important. The well-developed lumbar affections are associated with an early stage (case 1) or a hardly developed sacroiliitis (case 2). These alterations cannot be recognized, especially in the second case, by traditional radiological methods. Our observations accord with the data of DOUGADOS (1993), who describes cases of AS with well-developed syndesmophytosis and only clinically testable sacroiliitis.

Data on the prevalence of AS in modern populations are a little heterogeneous (they vary between 0.05 and 1%), due to racial differences (KELLGREN, 1964; SIMON et al., 1984; BAHK, 1994). A value of around 0.1% is most commonly quoted (RESNICK and NIWAYAMA, 1988). There is a clear male predominance, the disease affecting mainly male populations (SIMON et al., 1984; BAHK, 1994).

There is a strong association between AS and the presence of the histocompatibility antigen HLA-B27; the AS prevalence follows HLA-B27 in a

population (LE LOET and BRUNO, 1990; BAHK, 1994). Clinical studies reveal the association of the classical radiological and pathological changes of AS and several autoantibodies, in both HLA-B27-positive and negative patients (LAKOMEK et al., 1991). Whatever the correct etiological factor of the disease may be, its heritability (the hereditary predisposition to AS) has already been justified (RESNICK and NIWAYAMA, 1988; GÖMÖR and BÁLINT, 1989). Different literature data suggest that the family incidence of AS is 10 to 100 times higher than its incidence in a normal population (KELLGREN, 1964; RYCKEWAERT, 1980; SIMON et al., 1984).

Although the relatively rare case-histories on AS in the paleopathological literature (ORTNER and PUTSCHAR, 1985) are not sufficient to allow calculation of a real prevalence of the disease in ancient times, its frequency does not seem to have been higher than today. The results of previous studies revealed the prevalence of SNSA in adult populations, which was diagnosed in historical series to be either 0 or a value below 1% (BÉRATO et al., 1990; PÁLFI, 1990, 1993; MOLNÁR and MARCSIK, 1994).

It is a little surprising that two well-developed cases were found during examinations of 83 skeletons from Bácsalmás. Analysis of the 54 adult skeletons only clearly showed that the prevalence of AS in the adult population is relatively high, reaching even 3.7%. With regard to the low number of observed cases, we would like to avoid drawing further conclusions. The location of the two affected skeletons in the cemetery is most interesting: the skeletons (Nos 80 and 90) were found in two neighboring graves (WICKER, manuscript). The importance of this fact should not be overestimated, of course, but the neighboring graves together with the hereditary predisposition of the disease and the striking resemblance between the morphology and patterns of the lesions, suggest that there may have been a family relationship between the two individuals. Further biomolecular or paleoserological examinations may clarify this problem.

Acknowledgements

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PROBABLE CASES OF SKELETAL INFECTIONS IN THE 17TH CENTURY ANTHROPOLOGICAL SERIES FROM BÁCSALMÁS (HUNGARY)

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Abstract

A paleopathological study was carried out on human skeletal remains from a 17th century cemetery at Bácsalmás, and revealed three probable cases of skeletal infections.

The first one involves a male skeleton (grave No. 39), which presents signs of a severe infectious disease. The destructive lesions and some new-bone formation on the cervical, thoracic and lumbar vertebrae point to multifocal spondylodiscitis. The second case (grave No. 61) involves a young male skeleton, with a mild form of spondylodiscitis on 9 thoracic vertebral bodies, and serious osteolytic lesions and periosteal new-bone formation on 9 right side ribs. In the third case, two fragments of soft tissue calcifications were found, among the well-preserved remains of a male skeleton (grave No. 85). The morphology and the locations of the formations indicate pathological pleural calcifications.

The ankylosis of 3 right side ribs of the same skeleton should be mentioned.

The above-mentioned alterations indicate different types (hematogenous and direct spread) of skeletal infections. Skeletal tuberculosis is discussed as a possible common infection.

The differential diagnosis, which is based primarily on macroscopic methods, will be complemented by further histological and molecular biological methods.

Key words: paleopathology, 17th century, skeletal infections, tuberculosis.

Introduction

Infection of the skeletal tissues results from microbial organisms that are either bloodborne (hematogenous infection) or implanted directly into the bone. Even though bone is a very dynamic and sensitive tissue, relatively few infectious diseases produce recognizable lesions (KELLEY, 1989). Some infectious conditions, however, do affect the skeleton and careful analysis can reveal much about human adaptation in response to disease. Unfortunately, many of the infectious diseases produce morphologically overlapping responses in skeletal tissues. These responses make specific diagnosis difficult, especially in ancient bones (ORTNER and PUTSCHAR, 1985).

In spite of these difficulties, paleopathologists have made significant contributions to osteopathological diagnosis by studying dry bone remains from modern and ancient collections (e.g. MOLLER-CHRISTENSEN, 1961; HACKETT, 1976; STEINBOCK, 1976; KELLEY and EL-NAJJAR, 1980; MANCHESTER, 1984; ORTNER and PUTSCHAR, 1985; KELLEY, 1989; BUIKSTRA and WILLIAMS, 1991). The

paleopathological detection of radiologically invisible subtle bone lesions (such as the subtle periostitis of internal rib surfaces associated with pulmonary tuberculosis presented by KELLEY and MICOZZI (1984)) can promote a clinical diagnosis by providing new data.

The aim of the study of skeletal infections in paleopathology is not only the differential diagnosis of the detected lesions, but also research into the origin, evolution and spread of the infectious diseases. The present paleopathological analysis forms part of an international research program, focused on studies of the paleoepidemiological conditions of infectious diseases, based essentially on the historical anthropological collection of the Department of Anthropology at József Attila University.

Materials and Methods

The 91 graves in the Bácsalmás-Homokbánya cemetery were excavated in 1993, under the direction of ERIKA WICKER, archeologist and director of the Thorma János Museum at Kiskunhalas. The coins found in some graves revealed that the cemetery was in use during the 17th century (WICKER, manuscript). Historical data helped with the identification of the excavated cemetery (IVÁNYI, 1909; BOROVSZKY, 1910). It was used by a Serbian community living at Bácsalmás during the Turkish occupation of Hungary (WICKER, personal communication).

The subject of the anthropological and paleopathological analysis consisted of 83 skeletons, which are to be found in the collection of the Department of Anthropology at the József Attila University. The majority of the skeletal material is in a good or medium state of preservation.

The sex and age at death were determined with traditional methods used in historical anthropology (FEREMBACH et al., 1979; KNUSSMANN, 1988). The differential diagnosis was based on macromorphological methods, using both clinical and paleopathological special literature (e.g. ORTNER and PUTSCHAR, 1985; SILVERMAN, 1985; RESNICK and NIWAYAMA, 1988).

Results and Discussion

During the paleopathological study of the 83 human skeletal remains, besides other pathological conditions (traumas, degenerative joint diseases, spondylarthropathies, etc.), skeletal lesions due to probable infectious diseases could be detected in three cases. The three descriptions and discussions are presented separately.

Case No.1: Grave No.39. Male skeleton. Mature adult; relatively good state of preservation.

Description:

The most important pathological alterations of the skeleton are the erosive lesions of the vertebral bodies. The character of the lesions shows a considerable similarity: multifocal, circular, oval coalesced or irregular lytic areas in the vertebral bodies. Smooth bone resorption and small sinuses prevail; neural arch segments are uninvolved. The lesions have a maximum diameter ranging between 3 and 25 mm.

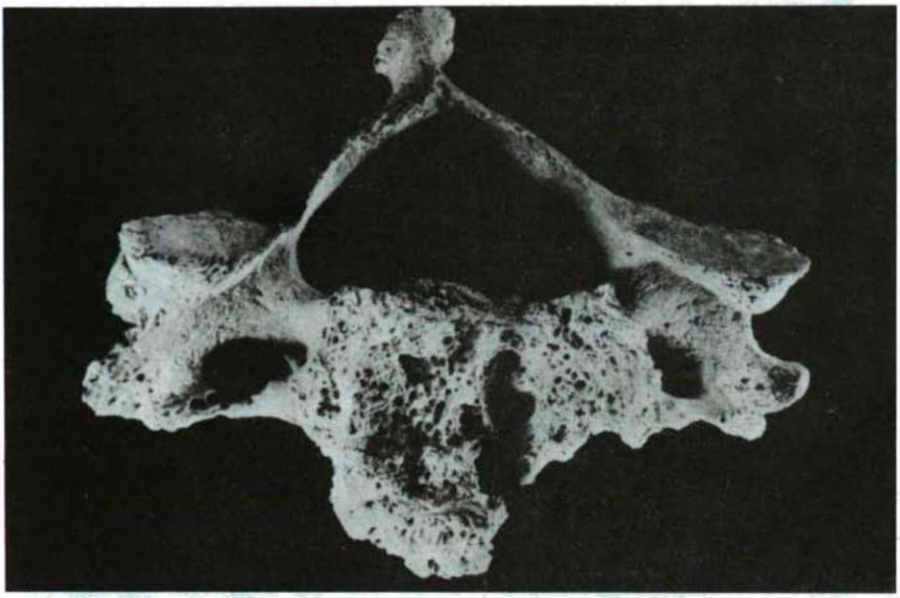

Fig. 1. Osteolytic destruction of the 4th cervical vertebral body. (Case No. 1: Grave No. 39)

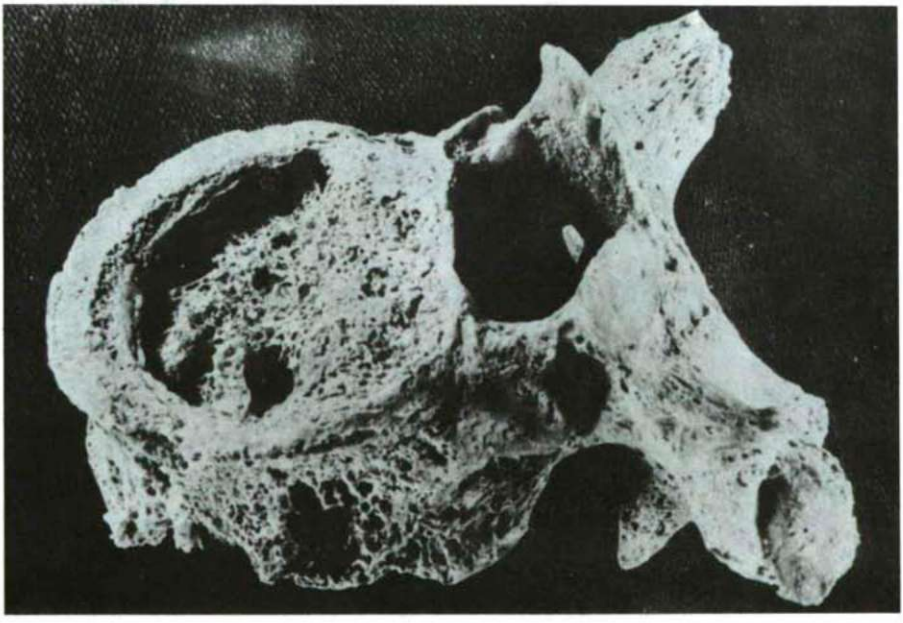

Fig. 2. Osteolytic foci in the 9th thoracic vertebral body. (Case No. 1: Grave No. 39)

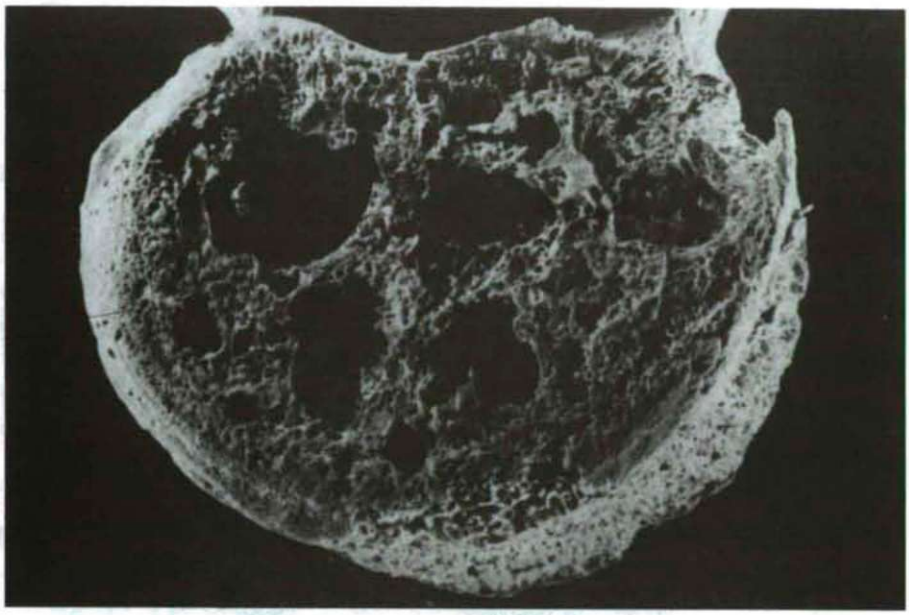

Fig. 3. Multifocal osteolytic destruction in the 2nd lumbar vertebra. (Case No. 1: Grave No. 39)

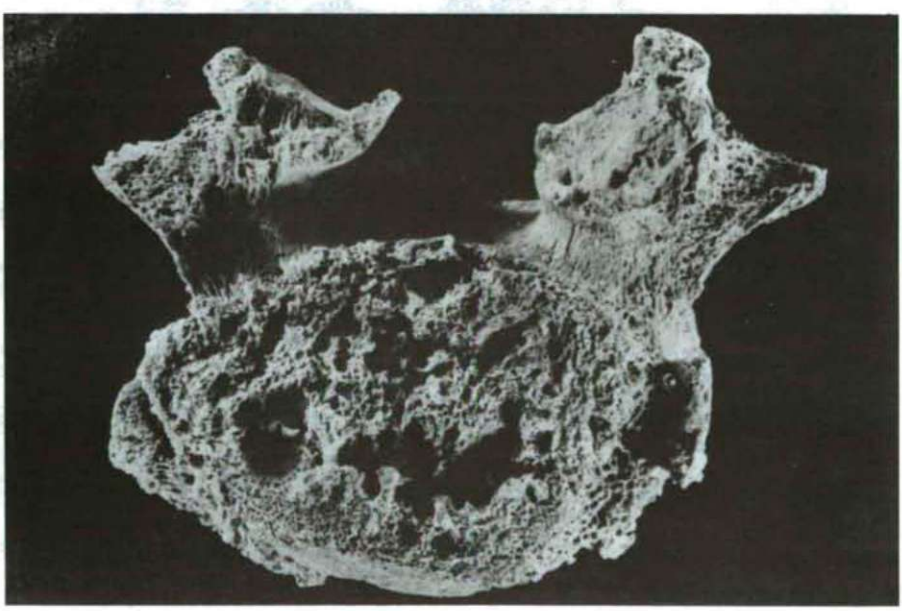

Fig. 4. Erosive and proliferative lesions on the 5th lumbar vertebra. (Case No. 1: Grave No. 39)

The lesions affect the vertebral column in several locations:

Cervical spine: two involved segments (C3-C4 and C4-C5) can be identified. The most important damage affects the 4th cervical vertebra: the bulk of the vertebral body is destroyed by the lytic process (Fig. 1). There is a little bony regeneration and remodeling of the trabecular system.

Thoracic spine: osteolytic involvement of the vertebral bodies between T5 and T11. Resorptive areas are to be found in the central portion of the body, sometimes extending to the antero-superior or antero-inferior margins. In some cases, small sinuses perforate the vertebral bodies and open in the lateral or anterior surfaces (Fig. 2). The most advanced stage of the alteration is to be seen in segments T8-T9.

Lumbar spine and lumbo-sacral border: two important foci can be differentiated. The first affects segments L2-L3 in the form of multifocal osteolytic destruction of the adjacent vertebral bodies (Fig. 3). There is a little bony regeneration around some lytic foci and slight periosteal and osteophytic development on the bodies. The second focus of advanced stage involvement is the lumbo-sacral border. There is massive osteolytic destruction of the adjacent bodies in segments L5-S1 and slight marginal osteophytic formation (Fig. 4).

For the purpose of diagnosis, it is important that slight periosteal new-bone formation can be observed on the distal part of the two tibiae, fibulae and calcaneums. The right side tibia shows signs of thickening and pathological axial deformation. With the exception of the above-mentioned alterations, there is no evidence of other infection or trauma and the bones do not exhibit any signs of demineralization. Other pathologies in case 1 are caries of the left maxillar first molar and spondylolysis of L5 (Fig. 4).

Discussion:

Although the *in vivo* process is evident in the bone-forming reactions (periosteal or osteophytic new-bone formation), in the case of osteolytic reactions it is difficult to exclude taphonomic effects. In the present case, the limited form of the lesions, the slight sclerotic margin of the lytic areas and the reactive new-bone formation suggest an evident *in vivo* process (ORTNER and PUTSCHAR, 1985).

The localized alterations (dental caries and spondylolysis) reveal independent pathological conditions. As far as the alterations of the lower limb bones are concerned, it is known that several pathological conditions may be associated with periosteal bone reactions. In this case, the morphology of the lesions suggest that a post-traumatic pyogenic infection is most likely (BULLOUGH and VIGORITA, 1984).

The above-mentioned spinal lesions are easily distinguishible from degenerative, traumatic or inflammatory (spondylarthropathies) processes (BUIKSTRA, 1976; KELLEY and EL-NAJJAR, 1980; ORTNER and PUTSCHAR, 1985). Metastatic carcinomas can produce resorptive foci in vertebral bodies, but they are frequently associated with neural arch and rib lesions and the intervertebral discs are not affected (LAPIS, 1989); consequently, they can be excluded in our case.

The destructive lesions and some new-bone formations on the cervical, thoracic and lumbar vertebrae reveal multifocal spondylitis with a probable hematogenous

spread (HORVÁTH and FORGÁCS, 1984; BAHK, 1994). Within the group of spinal infections, a large series of microorganisms can produce similar pathological alterations. Resorptive foci on the vertebrae are not infrequent in the skeletal involvement of actinomycosis, tuberculosis, pyogenic osteomyelitis, coccidioidomycosis or blastomycosis (DE SEZE and RYCKEWAERT, 1983; RESNICK and NIWAYAMA, 1988; KELLEY, 1989). In the rare cases of osseous involvement due to actinomycosis, the mandible is the most commonly affected element; neural arch lesions occur as frequently as those of the body, and the disk space is maintained (BUIKSTRA, 1976; ORTNER and PUTSCHAR, 1985).

In skeletal tuberculosis, which is the most probable diagnosis in this case, the most common lesion is tuberculosis of the vertebral bodies and intervertebral discs, or tuberculous spondylodiscitis (MARTINI, 1988). Although the classical and relatively easily recognizable form in advanced Pott's disease is angular kyphosis with ankylosis of the vertebral bodies involved, in active tuberculous spondylitis, erosive lesions, smooth bone resorption and osteolytic changes of the subchondral region of the vertebral body are most frequently seen (MARTINI and OUAHES, 1984; KELLEY 1989). The study by KELLEY and EL-NAJJAR on documented cases of skeletal tuberculosis revealed a considerable variability of the vertebral lesions (KELLEY and EL-NAJJAR, 1980).

The anatomo-pathological changes of hematogenous pyogenic spondylitis are very similar to those of skeletal tuberculosis. Unlike pyogenic bone infection, tuberculosis affects the spine more frequently than the long bones. Tuberculous spondylitis commonly involves multiple vertebrae, whereas pyogenic osteomyelitis always involves two neighboring vertebrae only (BAHK, 1994).

In addition to the above-mentioned diseases, fungal infections can also be implicated in the differential diagnosis of tuberculosis. Of the fungal infections, blastomycosis and coccidioidomycosis are perhaps the most difficult to distinguish from tuberculosis in human skeletal remains. Bone involvement, in the form of multiple resorptive foci, is frequent in the disseminated form of both diseases (RESNICK and NIWAYAMA, 1988).

Case No. 2: Grave No. 61. Male skeleton. Young adult; relatively good state of preservation.

Description:

The relatively robust skeleton of a 20 to 25-year-old male is in a good general state, without any signs of demineralization. Pathological alterations are seen exclusively on the bone remains of the thorax.

Ribs (right side): The most characteristic lesions are the diffuse periosteal new-bone formation on 9 (4th to 12th) of the right side ribs. The lesions affect predominantly the visceral surface of the ribs. Morphologically, these lesions consist of plaques of new bone with discrete pits indicating the inflammatory reaction of the periosteum. Focal lytic lesions, ranging from 5 to 35 mm, are found on the pleural aspects of the 5th to the 10th ribs (Fig. 5a-d). In some cases, the osteolytic lesions are

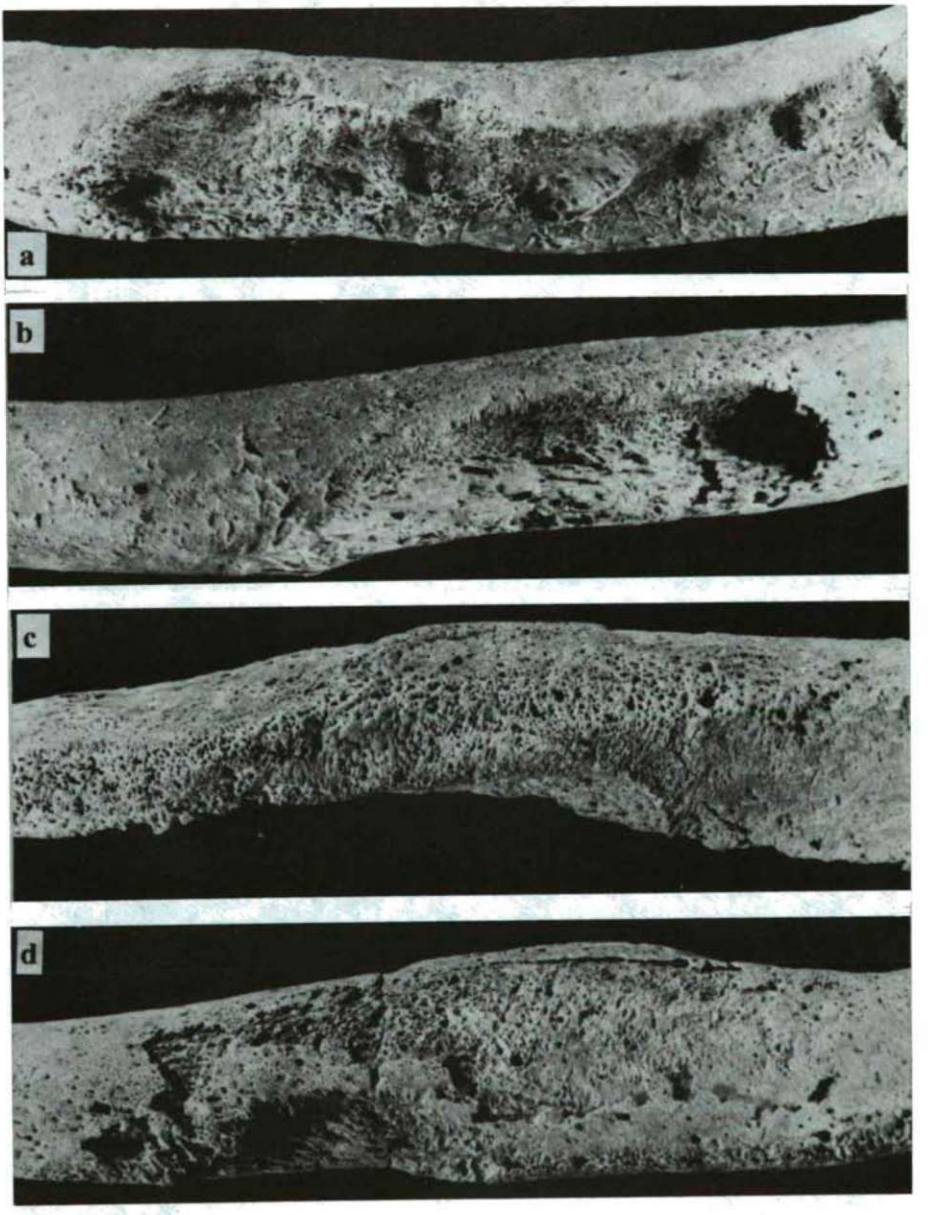

Fig. 5a-d. Periostitis and osteolytic lesions on the visceral surface of the 6th (a), 7th (b), 9th (c), and 10th (d) right ribs. (Case No. 2: Grave No. 61)

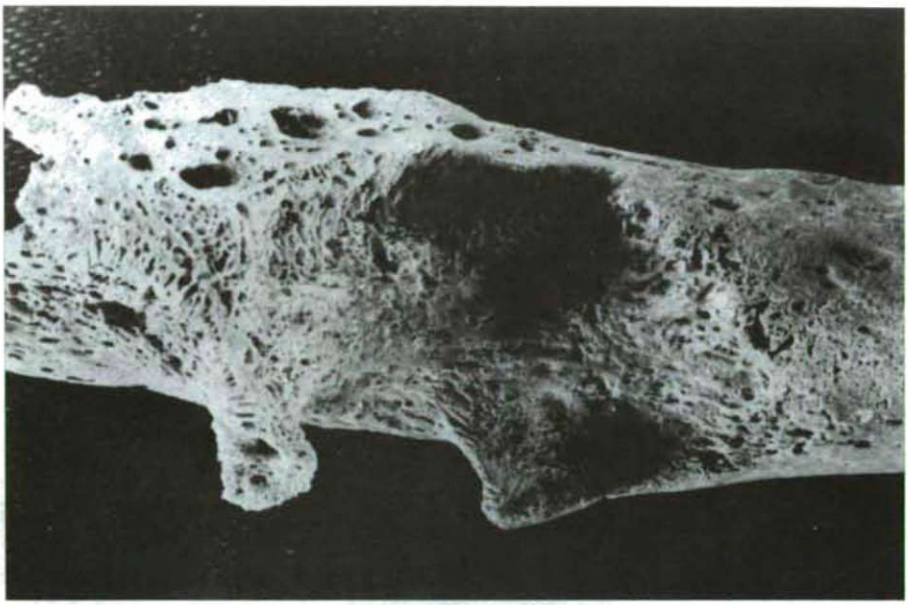

Fig. 6. Reactive new-bone formation around an osteolytic focus of the 5th right rib. (Case No. 2: Grave No. 61)

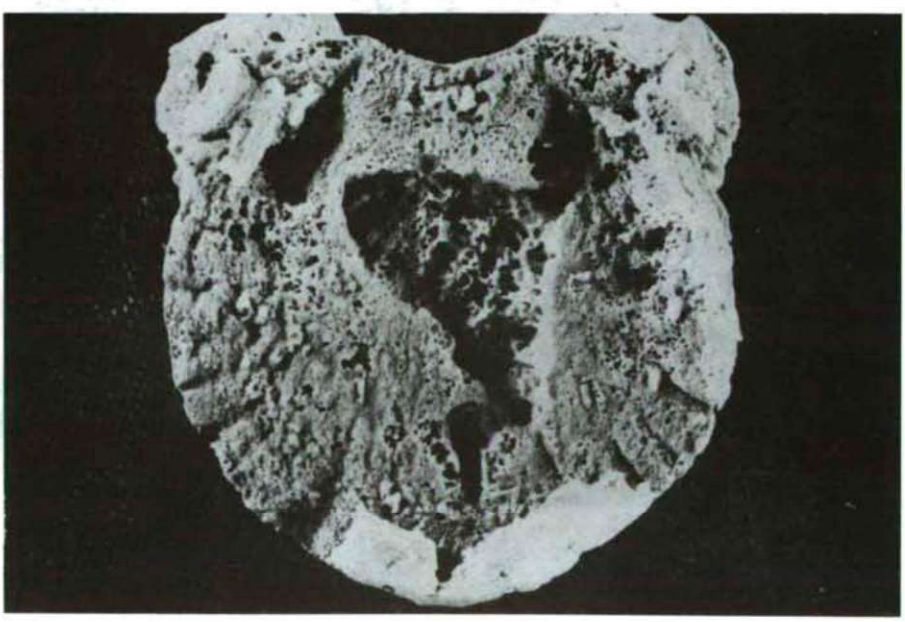

Fig. 7. Irregular lytic lesion on the 6th thoracic vertebral body. (Case No. 2: Grave No. 61)

surrounded by osteoblastic formation. On the inner surface of the lytic cavities, fine reactive new-bone formation and remodeling of the ancient trabecular bone can be seen (Fig. 6). Pathological changes can be detected not only in the subperiosteal and cortical region: the subcortical trabecular bone is completely remodeled in several localizations.

Right clavicle: the bone is thickened and quasi-completely covered by periosteal new-bone formation.

Thoracic spine: Irregular lytic areas are present on the adjacent vertebral bodies of segments T4 to T12. Their extent varies between 4 and 25 mm; the maximal depth reaches 7 mm (Fig. 7). Very discrete new-bone formation can be detected in some of the lytic foci.

Discussion:

In spite of the difficulties in the explanation of periostitis in paleopathology, the observed periosteal new-bone formation on the right side clavicle and ribs suggests infectious conditions (BULLOUGH and VIGORITA, 1984; ORTNER and PUTSCHAR, 1985; RESNICK and NIWAYAMA, 1988). The osteolytic alterations of the ribs (evidently due to an in vivo process, which is justified by the detected sclerotic new-bone formation) also indicate an osteitis of infectious origin (BRAUNER et al., 1982). Osteolytic alterations of the ribs are frequently seen in some tumorous and tumor-like conditions, e.g. in multiple myeloma or osteolytic metastatic carcinoma (ORTNER and PUTSCHAR, 1985). In this case, howewer, the morphology, the skeletal pattern of the lesions and the lack of typical locations of myeloma or carcinoma metastases (skull, pelvis, vertebral bodies, proximal femoral epiphyses, etc.) do not favor such a diagnosis. Subchondral erosions of the vertebral bodies could suggest the presence of SCHMORL'S nodes, but their irregular aspects, their considerable dimensions and the presence of reactive new-bone formation point to a more likely early-stage spondylodiscitis (MARTINI, 1988).

As for the location of the lesions of the thoracic cage, the asymmetric (unilateral) character of the rib lesions suggests that a direct infectious process is more probable than a hematogenous infection. In the case of the detected vertebral lesions, a hematogenous spread seems more possible.

There are relatively few references about periosteal rib lesions in radiological or pathological diagnostic reports on infectious diseases (KELLEY and MICOZZI, 1984). This fact results from the often very subtle character of these alterations (ROBERTS et al., 1994). Osteomyelitis of the ribs, in the form of geode-like cavities, solitary or multiple lytic lesions, is reported in cases of actinomycosis, blastomycosis, coccidioidomycosis, typhoid or paratyphoid osteomyelitis, pyogenic osteomyelitis, syphilis and skeletal tuberculosis (BRAUNER et al., 1983; DE SEZE and RYCKEWAERT, 1983; MARTINI, 1988; RESNICK and NIWAYAMA, 1988). The lack of specific diagnostic criteria (e.g. typical cranial or tibial lesions in treponematosis and lumbar affection in typhoid or paratyphoid osteomyelitis) and the pattern of the observed lesions help to exclude some of the mentioned diseases.

The unilateral infections of the ribs in this case suggest the possibility of direct

extension from right side pleural and/or lung foci. Thus, the major diagnostic problem is to distinguish between the infectious processes which can provoke pulmonary diseases and may disseminate through the pleura to the ribs. The possibility of direct extension in rib lesions is described in cases of pulmonary tuberculosis, actinomycosis, nocardiosis, blastomycosis and coccidioidomycosis (RESNICK and NIWAYAMA, 1988). In all of these infections the osseous involvement of the ribs by direct spread is characterized by a combination of lysis and sclerosis. As the specificity of microbial methods used in modern clinical practice is much higher than that of radiological methods, insufficient data are available to differentiate these lesions on this basis. As far as the most widespread (and, from an epidemiological point of view, the most important) of these infectious diseases, human tuberculosis, is concerned, the differential diagnostic questions of periosteal rib lesions are neglected in the medical literature, because of the more frequent and more typical osteologic lesions due to the hematogenous infection (ROBERTS et al., 1994).

Several important pathological and paleopathological studies have been carried out during the past fifteen years. As a result, several of these questions have been clarified. KELLEY and EL-NAJJAR (1980) presented rib lesions which show a considerable degree of diversity, resemble our cases and come from a documented series of skeletal tuberculosis. Research by KELLEY and MICOZZI (1984) on the Haman-Todd osteological collection recorded that 39 (8.8%) of a total of 445 skeletons of people dying of tuberculosis exhibited periosteitis or evidence of localized abscess adjacent to the visceral surface of one or more ribs. The authors suggested that the rib lesions were a result of pulmonary tuberculosis. Other studies have also noted inflammatory lesions of the ribs from ancient and modern contexts (MOLTO, 1990; BUIKSTRA and WILLIAMS, 1991; PFEIFFER, 1991; ROSE and HARTNADY, 1991; WAKELY et al., 1991; ROBERTS et al., 1994). Tuberculosis was proposed in the differential diagnosis of these bone changes. In the most recent (and the most representative) of these studies, the authors investigated the frequency of periosteal new-bone formation on the visceral surfaces of the ribs of 1718 individuals from the Terry collection (ROBERTS et al., 1994). Their results demonstrate that rib lesions were much more common in individuals dying from tuberculosis (61.6%, or 157/255) than in individuals dying from other causes (15.2%, or 165/1086).

Taking into consideration the diagnostic criteria, the case histories and epidemiological data found in the medical and paleopathological literature, it can be concluded that in case 2 the rib lesions suggest pulmonary infection. Although the pathological changes of the visceral surfaces are consequences of a possible direct spread through the pleura, the hematogenous spread from a pulmonary focus cannot be excluded because of the spondylodiscitis of the thoracic vertebrae. Direct infection and/or hematogenous spread can result in inflammatory lesions of the right clavicle.

Fig. 8. Ankylosis of the 7th to 9th right ribs in the area of the costotransverse articulations. (Case No. 3: Grave No. 85)

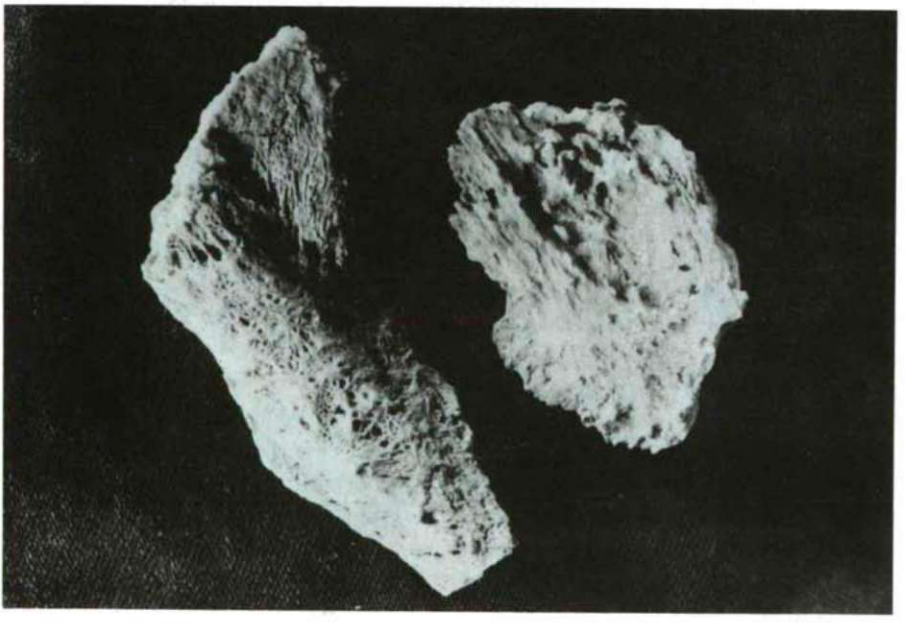

Fig. 9. Soft tissue calcifications suggesting pleural plaques. (Case No. 3: Grave No. 85)

Case No. 3: Grave No. 85. Male skeleton. Mature adult; very good state of preservation.

Description:

This case involves the well-preserved skeleton of a 50-60-year-old man.

The quasi-complete and very robust skeleton displays a good state of mineralization. There is dental caries in the left lower third molar. In spite of enthesopathic signs in the areas of some muscular insertions (calcaneums, pelvis and some vertebral bodies), which are not sufficiently developed to allow a diagnosis of diffuse idiopathic skeletal hyperostosis, there is no sign of a generalized pathological condition.

However, two surprising phenomena must be mentioned. The first is the ankylosis of 3 right side ribs (Fig. 8). The bony fusion affects the 7th to 9th right ribs, in the area of the costotransverse articulations. The superficial new-bone formation on the visceral surface should be mentioned. The process is unilateral and exclusively localized in this area. The other bones of the complete and well-preserved thoracic cage do not present any pathological lesions.

The second interesting phenomenon is the presence of soft tissue calcifications. Among the osseous remains of the rib cage of the skeleton, two pathological formations were found (Fig. 9). (We have no more valuable locations of this calcified material from an archeological context.) The dimensions are as follows: 62 mm x 30 mm x 3-4 mm; and 62 mm x 25 mm x 4-6 mm. Macroscopically, they are hard and flat, with a slight curvature of the longer fragment. Their surface is irregular, and some lamellar character can be recognized.

Discussion:

Pathological changes of costotransverse and costovertebral joints can occur (relatively rarely) in certain rheumatologic or metabolic diseases. There are several literature references to inflammatory changes and ankylosis in these locations in seronegative spondylarthropathies. In cases of advanced stage diffuse idiopathic skeletal hyperostosis, the costotransverse ligaments may be ossified, but the articular surfaces are not affected (RESNICK and NIWAYAMA, 1988). In these conditions, however, the diseases affect predominantly other parts of the skeleton, and the processes affect the ribs bilaterally.

Localized ankylosis of bones or joints is more likely following traumas or localized infection (ORTNER and PUTSCHAR, 1985). In the present case, the latter possibility seems more probable because of the location of the alterations. There is no evidence, however, of fracture or other localized traumas in the affected area. The superficial, reactive new-bone formation on the visceral face of the ankylosed ribs in the affected area suggests an inflammatory reaction due to a probable infection. The localized character reveals an infection from some neighboring focus.

The morphology and the location of the calcified formations indicate pathological pleural calcifications. These cases are very similar to the well-documented cases of pleural plaques presented in the paleopathological literature

(KRAMAR, 1984; BAUD and KRAMAR, 1991). In those cases, the authors dicuss the possibility of the infectious or traumatic origin of the calcifications. In our cases, the lack of traumatic processes makes the former version more acceptable.

The pathological and radiological literature indicate that calcifications of the pleura appear following chronic pleurisy, especially in cases associated with pulmonary tuberculosis (LAPIS, 1989). In primary tuberculosis, the necrotic foci in any component (lung or pleura) heal by progressive fibrosis, hyalinization and calcification. Pleurisy is very common in primary tuberculosis and large free pleural effusions may develop during the infection. The localized thickenings and the secondary calcifications may persist until late in the disease (SILVERMAN, 1985). From paleopathological material, ZIAS (1991) presents cases of calcified pleura, which are interpreted as evidence of tuberculosis.

In case 3, we can suggest a correlation between the two observed alterations, due to a presumed pulmonary infection.

Conclusions

During the paleopathological study of human skeletal remains from the 17th century cemetery at Bácsalmás, three probable cases of skeletal infections have been identified.

In case 1, the morphology, location and pattern of the vertebral lesions suggest (similarly as for other cases reported in the paleopathological literature (BUIKSTRA, 1976; STEINBOCK, 1976; KELLEY and EL-NAJJAR, 1980; KELLEY, 1989; BÉRATO et al., 1991; POWELL, 1991)) a diagnosis of an active multifocal tuberculous involvement. On the basis of morphological investigations, however, the possibility of an atypical disseminated pyogenic infection (possibly correlated with the observed lower limb alterations) or a disseminated fungal disease, cannot be ruled out.

The periosteal and erosive rib lesions discussed in case 2 suggest a pulmonary infection. In addition to the assumed direct spread through the pleura to the ribs, hematogenous infection can also be presumed. The literature data indicate that the most probable cause of such lesions is pulmonary tuberculosis (KELLEY and EL-NAJJAR, 1980; KELLEY and MICOZZI, 1984; PFEIFFER, 1991; ROBERTS et al., 1994). Actinomycosis or some fungal infection, such as blastomycosis or coccidioidomycosis, also have to be taken into consideration (RESNICK and NIWAYAMA, 1988; MOLTO, 1990).

The rib lesions and especially the pleural calcifications seen in case 3 are probable consequences of a pulmonary infection. Special literature data mention the possibility of these alterations in different cases of tuberculosis (SILVERMAN, 1985; LAPIS, 1989; BAUD and KRAMAR, 1991). We know of no references describing similar lesions as a result of other infections mentioned above. As the examined reactions are secondary signs of a primary pulmonary infection, we cannot exclude, at least theoretically, the possibility of a primary fungal infection.

The results of the present paleopathological diagnostic study therefore suggest that tuberculosis is the most probable common cause of the infectious conditions described.

Finally, it proved possible to understand the limits of morphological studies in the paleopathological diagnosis of infectious diseases. It seems probable that further macroscopic investigations of the presented lesions will not clarify the etiological questions of these lesions further. The use of biomolecular analysis of bone for mycobacterial DNA in appropriate skeletal material, as to be seen in some recent publications (SPIGELMAN and LEMMA, 1993; DIXON et al., 1994), may promote further discussion.

The aim of this work was to attract attention to the possibility of the identification of "atypical" forms of skeletal tuberculosis. The prevalence of tuberculosis in archaic human skeletal remains is traditionally based on the paleopathological diagnosis of Pott's disease of the spine, or pathological changes in major weight-bearing joints of the body (e.g. FORMICOLA, 1987; DUTOUR et al., 1991; STIRLAND and WALDRON, 1989; STROUHAL, 1991), although any bone of the body can be affected by a considerable range of tuberculous lesions. This phenomenon characterizes the results of our research in Hungary: during the previous diagnostic studies, only the "classical" cases were taken into consideration (MARCSIK, 1972; FARKAS et al., 1976; PÁLFI, 1991; PÁLFI et al., 1992; MARCSIK and PÁLFI, 1993; MARCSIK et al., 1994). It seems necessary to reexamine the previously detected inflammatory rib lesions, for example in the case published by ÉRY (1982).

In order to be able to carry out a better paleoepidemiological analysis, we must examine further historical populations, and develop the morphological diagnosis via the histological and molecular biological methods mentioned above.

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Short communication

GROWTH AND ETHYLENE EVOLUTION OF TISSUE CULTURES IN PRESENCE OF NITRITE

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It was earlier established (ZSOLDOS et al., 1993) that the growth of wheat roots and shoots is inhibited by nitrite in the uptake solution. However, little information is available on the effects of nitrite on other kinds of growth. The experimental conditions in in vitro cultures are much more controlled, but the cells are not organized as in in vivo tissue. Thus, correlative influences are excluded. Tissue cultures are suitable and useful objects for the study not only of cell enlargement, but also of cell division.

The present paper reports on the effects of nitrite on in vitro callus cultures of *Nicotiana tabacum* cv. Petit Havana, SR1. The calli were grown on Murashige-Skoog basal medium (MURASHIGE and SKOOG, 1962) containing 0.2 μM kinetin, 17.14 μM indole-3-acetic acid and 0.45 μM 2,4- dichlorophenoxyacetic acid. The medium also contained nitrogen in the form of NH_4NO_3 (20.6 M) and KNO_3 (18.79 M), and nitrite was added to the medium in a concentration of 0.4-9.4 mM in the form of KNO_2. The fresh weight was measured on day 21 after inoculation.

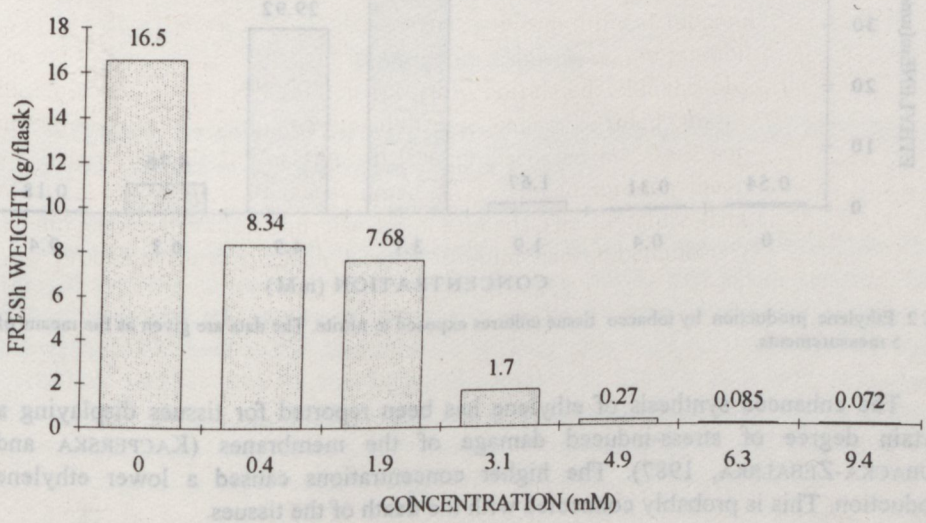

Fig. 1. Effects of nitrite on the growth of *N. tabacum* tissue cultures. Values presented are the means of 7-10 measurements.

Nitrite strongly inhibited the growth and the proliferation of the cultures (Fig. 1). When only 0.4 mM nitrite was present in the medium (this concentration is equivalent to 1/50 of the nitrate in the MS medium), the growth of the cultures was inhibited to an extent of about 50% as compared with the control (without nitrite), and there was no proliferation in the presence of 4.9 mM nitrite.

Nitrite could be considered a kind of stress factor. In addition to other physiological functions, ethylene, a gaseous plant hormone, is associated with a wide variety of stress responses in higher plant cells, too. Earlier experiments indicated that ethylene production by tobacco calli exhibits a slight peak on the 6th-8th day after inoculation (KÖVES and SZABÓ, 1987). The ethylene evolution of 7-day-old calli grown in basal medium containing different concentrations of nitrite was measured by gas chromatography at 80 °C with a flame ionization detector. The culture flasks were sealed by gas-tight caps until measurement. The total accumulation of ethylene was determined after a 24-h incubation. On increase of the nitrite concentration of the medium, the ethylene production slightly rose up to 1.9 mM nitrite (Fig. 2). The ethylene evolution suddenly multiplied for calli grown on medium containing 3.1 mM nitrite. This nitrite concentration resulted in a significant decrease in the growth of the calli (Fig. 1).

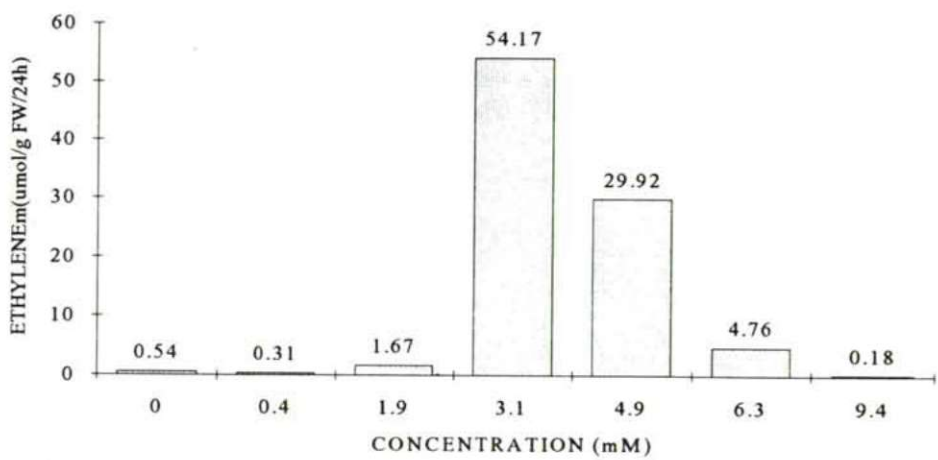

Fig. 2. Ethylene production by tobacco tissue cultures exposed to nitrite. The data are given as the means of 5 measurements.

The enhanced synthesis of ethylene has been reported for tissues displaying a certain degree of stress-induced damage of the membranes (KACPERSKA and KUBACKA-ZEBALSKA, 1987). The higher concentrations caused a lower ethylene production. This is probably connected with the death of the tissues.

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Short communication

NEW AUCHENORRHYNCHA SPECIES IN HUNGARY: *CHLOOTHEA ZONATA* EMELJANOV, 1959

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Introduction

EMELJANOV described the *Chloothea zonata* species (Homoptera, Cicadellidae) from Kazakhstan in 1959. At that time he considered that the appearance of the species depended on the presence of grasses, and primarily *Festuca sulcata*. Later (EMELJANOV, 1964), he mentioned the *Stipa lessingiana* and *Stipa sareptana* species as host plants. Since the description, the distributional data have been extended towith the Southwest of the European part of the former Soviet Union (EMELJANOV, 1964), then to Mongolia, and the Altaj Mountains (NAST, 1972).

Hungarian biotopes

In Hungary we collected the leafhopper in Kéleshalom in 1991, and in Fülöpháza in 1992-93 , altogether 131 specimens (Tables 1 and 2).

Kéleshalom:

The area is situated south of Kiskunhalas, near to the village of Kéleshalom. This sand-hill territory is covered by mosaics of grass, forest, and shrub patches. The different successional stages of the sandy grassland plant communities can be investigated very well in this conservation territory. We took insect samples in eight 200-300 m^2 patches with Barber traps and sweep nets. Phytocoenological data were collected as well. The *Chloothea zonata* leafhoppers were found in the samples from the perennial open sandy grassland (*Festucetum vaginatae danubiale*) and from the more closed grassland (*Festucetum vaginatae salicetosum rosmarinifoliae*).

Fülöpháza:

This area comprises part of the Kiskunság National Park. The soil is extremely dry, limy, wind-blown sand. The typical plant community is *Festucetum vaginatae*

stipetosum. At the beginning of summer *Stipa sabulosa*, and at the end of summer *Stipa capillata* is the main plant species of the upper grass layer. Barber and pan traps were used in spring, summer and autumn.

Table 1. The relative cover values of the more important plant species in the investigated biotopes

1991, 1992 August	Kélesh.1.	Kélesh.2.	Kélesh.3.	Kélesh.4.	Kélesh.5.	Fülöpháza
Festuca vaginata	62	33	20	52	28	16
Stipa spp.	10	25	30	2	8	37
Fumana procumbens	6	1	12	11	0	4
Alyssum tortuosum	3	0	1	2	0	12
Euphorbia sequieriana	8	2	1	4	0	8
Teucrium chamaedris	0	10	0	0	14	0
Plant coverage	16	35	39	32	52	38
No. of plant species	18	17	17	16	20	20

Table 2. The individual number and relative frequencies of *Chloothea zonata* in the different biotopes

	trap	No. of individuals	relative frequency	relative frequency of imagos
Kéleshalom				
I. 07.27-08.14.	Barber	4	8.7	15
II.07.27-08.14.	Barber	12	29.7	78
III.07.27-08.14.	Barber	2	6.5	18
IV.07.27-08.14.	Barber	3	3.6	14
V. 07.27-08.14.	Barber	2	5	22
Fülöpháza				
1993.05.14-28.	Barber	40	53	0
1992.08.10-24.	Barber	13	65	81
1992.08.10-24.	Pan	58	57	64
1992.10.12-26.	Barber	1	3.1	3.3

Factors affecting the distribution of the species

From the relative cover values of the predominant plant species of the biotope and from the absolute cover values, it can be observed by means of classification (Renkonen index, weighted average) and ordination (PCA) methods that the distribution of the leafhoppers is determined by the presence of the *Stipa* species, together with the total cover of the vegetation, which affects the microclimate (Figs 1 and 2).

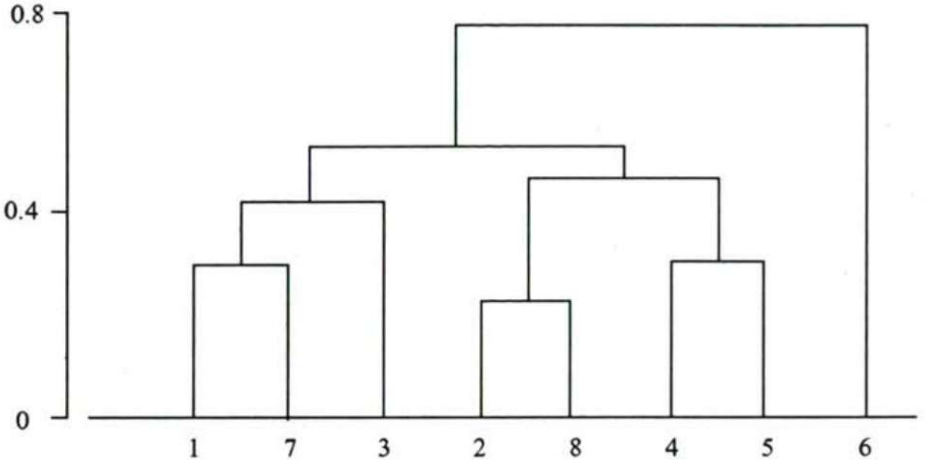

Fig. 1. Cluster analysis of the relative frequencies of *Chloothea zonata*, the cover of the important plant species, and the total plant cover. (1-6: plant species as in Table 1., 7: total plant cover, 8: relative frequency of the leafhopper)

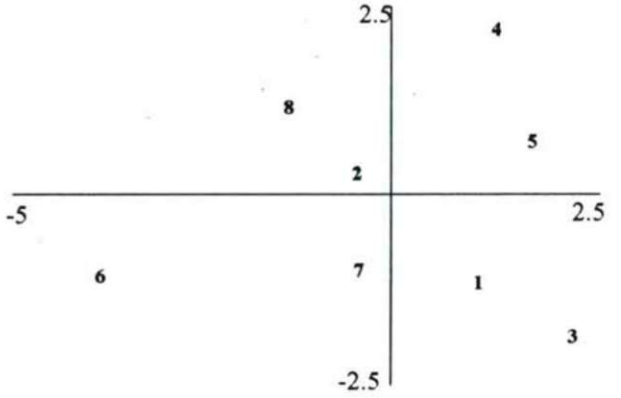

Fig. 2. PC-analysis of the relative frequencies of *Chloothea zonata*, the cover of the important plant species, and the total plant cover. The meanings of the numbers are the same as in Fig. 1.

Discussion

Chloothea zonata, a recently discovered species (genus) in Hungary, lives in sandy biotopes. It is the typical predominant species in the middle of summer, when otherwise the leafhopper community is rather poor in species. It probably spends the winter as a larva. The larvae can be found from spring until August. One generation

develops. Its appearance depends on the presence of *Stipa* plant species and also on the plant cover.

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MUSCARINERGIC AND NEUROPEPTIDERGIC RECEPTOR HETEROGENEITY AND SIGNAL TRANSDUCTION

D.Sc. Thesis

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Szeged
1992

Introduction and aims

The cells of multicellular organisms communicate by extracellular signals. The communication between these cells involves the following cellular events: 1. the synthesis of the neurotransmitter or hormone; 2. its release; 3. the binding of this transmitter or hormone to its receptors on the surface of the target cell membrane; 4. the change of the cellular metabolism; and 5. the removal of the signal, which often results in cessation of the response of the cell. This thesis provides details on the key elements of this communication: the perception and the transduction of the signal, the heterogeneity of the receptors and their role in the transfer of the signal. On the surface or inside the cells, the membrane receptors are complex molecular units which are able to bind neurotransmitters, hormones and other substances. These receptors are responsible for two functions. Firstly, through their high-affinity, specific binding, they are able to recognize and distinguish biologically active ligands; secondly, through their interactions with their coupling proteins, they make it possible to mediate the apropriate signals, which finally leads to the response. In neurons, the realization and joining of these two functions into one process is called neuronal signal transduction.

The muscarinergic (m1-m5), opioid (mu, delta, kappa, sigma, epsilon and lambda), and somatostatin receptors are transmembrane proteins. In the cell, when binding their high-affinity and selective agonists, they change their conformations and cause well-defined biochemical changes which can be inhibited by selective antagonists. These receptor types usually mediate well-characterized physiological functions and can be found in both the central and peripheral nervous systems and even in other non-neural tissues. They have a characteristic ontogenetic development, localization, and pharmacological and physiological features, with often a spectacularly demonstrable neuronal transport to the site of the physiological function. The number of neurotransmitter, neuromodulator and hormone receptors is currently known more than one hundred. For example, the receptor subtypes of the cholinergic

(muscarinergic M1-M5 and nicotinergic), opioidergic (mu, delta, kappa, sigma, epsilon and lambda), adrenergic (alfa1, alfa2, beta1 and beta2), dopaminergic (D1 and D2) or serotoninergic ($5HT_1$, $5HT_{1A, B, C}$, $5HT_2$ and $5HT_3$) systems are well known and also well characterized on a pharmacological basis, and the primary structures (the nucleotide sequences) of some of their genes are already known. This thesis includes discussion of the heterogeneity of the muscarinergic and opioid receptors and presents relevant results.

By the 1980s, the receptor hypothesis (relating to the conditions of binding of the ligand, the properties of the binding, and effects able to modulate the binding) and its common principles had been elaborated. The clarification of the conformational conditions of the receptor-ligand interaction started only afterwards, when the role of neuropeptides in signal transmission had been recognized. Several conformationally restricted peptide ligand are now known, and with their aid receptor-selective physiological effects can be produced. In many cases their application has made it possible to shed light on the role of the coupling proteins in signal transduction.

The elements of the endogenous opioid system are to be found in both the central and peripheral nervous systems; they mediate a number of physiological effects. By means of immunology and molecular biology, localization of the endogenous ligands of the opioid system revealed the distribution of this system in the brain. Since the first publication of the binding properties and regional distribution of the opioid receptors, twenty years ago, our knowledge has increased rapidly. On the basis of the primary structure three, and on the basis of the pharmacology six receptor subtypes can be distinguished, among them the mu, delta and kappa, which are well characterized from molecular biological and pharmacological respects, while the epsilon, sigma and lambda receptor subtypes are only sketchily known and their physiological roles have not been elucidated. Although several pharmacological and physiological investigations involving the use of classical (non-peptide) opioid ligands suggested the existence of the main subtypes (mu, kappa and delta), the results of these early findings have now been re-evaluated following the accessibility and use of highly effective and selective ligands.

With varying degrees of affinity, the endogenous opioid peptides (methionine-enkephalin [ME], leucine-enkephalin [LE], dynorphin, etc.) are capable of binding to several opioid receptors, and so they might be responsible for biological functions mediated by different receptor types. For example, the delta opioid agonist [D-Ala^2 or D-Leu^5]enkephalin is able to bind with a comparatively high affinity to the mu binding sites in the guinea-pig and rat brains. Obviously, for investigation of the physiological functions mediated by these opioid receptor subtypes, the need for highly selective ligands with high affinity and as resistant as possible to biodegradation is inevitable.

The active conformation of the linear oligopeptides (e.g. ME or LE) can at most be revealed at the moment of interaction with the receptor. However, through the use of suitable structural modifications (e.g. methylation or cyclization of the peptide sceleton), even the conformational and electrical conditions of receptor-ligand

interactions can be clarified. In the instance of the enkephalins, the application of cyclic structures proved suitable. In solution, such analogues have only a small number of conformations and, if the sceleton is rigid enough, during binding to the receptor their conformation does not change. Such a conformationally restricted cyclic antagonist is D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 (CTOP), with high affinity and mu selectivity. This analogue has a 32,500-times higher affinity for mu than for delta receptors, which practically means preclusion of the possibility of non-receptor-specific responses. Our knowledge concerning the physiology, pharmacology, and localization of the opioid receptors is based almost exclusively on the application of receptor-selective and very often conformationally restricted opioid ligands. This thesis discusses the results of pioneering investigations in which such a delta or a mu opioid receptor-selective conformationally restricted ligand was used.

The functions and features of any cell depend on the peptides inside the cell. However, the amounts of particular proteins are regulated by the function of the genes, i. e. the concentration of the mRNA coding the protein, the frequency of the translation and, of course, the stability of the protein. These three factors determine the scale and quality of the gene expression. The different activities of the genes and the control of the gene expression determine the functions and features of the cells. In eukaryotic cells, the control of the gene expression occurs decisively at the level of the transcription. The manifestations of the genes are determined by ontogenetic programmes, while the direction and the degree of any physiological process are determined by the unique process of gene expression. Some physiological or pathophysiological stimuli can increase the activity of certain genes and even the postranslational modifications of the primary transcripts can often be modified.

In the cell, the synthesis, release and degradation of the transmitters or the hormones, and also the number of receptors are regulated, and as a result of the gene function they are under genetic control. The transmitter substances of certain transmitter systems (cholinergic, catecholaminergic, aminergic, etc.), e.g. the acetylcholine, are synthesized in several reaction steps involving collaboration with a number of enzymes; further, their degradation can occur, and thus the regulation of transmitter synthesis involves control of the gene expression of some (catabolic and anabolic) enzymes. However, the synthesis of transmitter substances in the neuropeptidergic systems is under direct genetic control (transcriptional control, posttranslational modifications), and thus the regulation of gene expression in these systems can be investigated directly. The genes of these peptides, as sensitive indicators, can rapidly react to homeostatic or pathophysiological changes, while their gene expressions can be regulated differently even in a single cell. This thesis discusses results concerning regulation of the synthesis of two neuropeptides, vasopressin (VP) and prodynorphin (PD), and also Gs_α coupling protein. In connection with the metabolic and homeostatic changes during signal transduction, an account is further given of experience regarding the regulation of some trace elements and some mono- and divalent cations at the level of the nervous system.

There are three known families of endogenous opioid peptides: 1. the proenkephalin A derivatives of which the most important are the ME and LE derivatives, and other longer fragments such as the hecta- and octapeptide fragments and peptide E; 2. PD (proenkephalin B) -derived peptides such as the dynorphin A and B fragments, α- and β-neo-endorphin and leumorphin; 3. the proopiomelanocortin (POMC) -derived peptides e.g. α-, β- and γ-endorphins. Although the nucleotide sequences of these genes are similar to a certain extent, their expressions differ, and several physiological and pathophysiological factors may be involved in their control.

Beside its essential and well-characterized effects, VP seems important in memory processes and in the development of alcohol tolerance/dependence processes. The brain distribution of the cells expressing the peptide or rather the gene is well known; its localization in the brain has been revealed by immunocytochemistry and *in situ* hybridization studies. More and more details are available on the changes in VP level during altering environmental stimuli or experimental interventions, an also the regulation of its gene expression. Various factors are involved in this regulation. It was proved that, even in cells where PD and VP are colocalized, the regulations of their gene function differ considerably, even in the case of the same stimulus. The thesis discusses the changes in neuronal gene expression in the VP and PD genes during experimental intervention.

In certain signal transduction systems, the perception of the extracellular stimulus and the generation of the intracellular response are carried out by the same peptide or peptide complex. In other cases, the relay and the intracellular effector are different proteins, which are coupled to an intermediate guanine nucleotide binding regulator protein (G protein). The G proteins have a heterotrimeric structure (α, β and γ subunits); hardly a dozen Gs_α subunits are known, but they are able to bind nearly a hundred receptors and ion channels defined by pharmacological and/or molecular biological methods. The primary structures, the localization and the role in signal transduction of the different G proteins are known, as are the changes in the regulation of their genes during physiological and pathophysiological stimuli. The thesis reports investigations by *in situ* hybridization and *in vitro* transcription methods of the changes in the Gs_α expression during chroniç ethanol treatment.

There are a numerous methods suitable for the investigation of neuronal signal transduction. In investigations of the structure and the function of the receptors, use can be made of the registration of physiological, pharmacological (tissue or organ) and biochemical (receptor-induced activation of enzymes) responses or direct receptor-binding measurements (membrane binding, autoradiography). Through the use of the high-affinity and often conformationally restricted, receptor-selective ligands developed in the last few years, the receptor-specific physiological responses can be determined, and the data acquired with the use of the early ligands, which often had cross-reactions, can frequently be re-evaluated. The use of these ligands with high specific activity made it possible to measure their binding to the receptors directly. With the aid of the methods of molecular biology (*in situ* hybridization, *in vitro* transcription, analysis of gene sequences, Northern analysis and polymerase chain

reaction), it is possible to investigate the control of the synthesis of certain peptides, including neuropeptides, at the level of the genes.

This thesis investigates the signal transduction mediated by the different muscarinergic and neuropeptidergic (primarily opioidergic, somatostatinergic and vasopressinergic) receptors in neuronal tissue under normal, pathological or experimental conditions. Data acquired through the use of receptor-binding studies and the methods of molecular biology are summarized. An account is also given of the changes in the control of the gene functions of two neuropeptides (PD and VP) and a coupling protein (Gs_{α}) during experimental interventions.

In the course of the investigations, answers were sought to the following questions:

1. What are the characteristics of the synthesis, the intracellular transport, the subcellular and autoradiographic distributions of the mAChRs in the central and peripheral nervous systems of the rat?
2. How do the binding parameters of the mAChRs in the central and peripheral nervous systems of the rat change under physiological, pathophysiological and experimental conditions?
3. How can the muscarinergic, somatostatinergic and delta opioid receptors be characterized in the course of the neurodegenerative Alzheimer's disease? Do these binding parameters change in two animal models of Alzheimer's disease?
4. What are the characteristics of the heterogeneity of the mAChRs in the tissues of the heart and brain in the rat, and in human neuroblastoma cultures?
5. What are the conditions of receptor selectivity according to the ligand conformations in the case of substance P and the delta and mu opioid receptors?
6. What are the autoradiographic distributions of somatostatin, delta and mu opioid receptors in the central nervous system of the rat?
7. What are the physiological and pharmacological characteristics of DPDPE and CTOP?
8. How do the delta opioid receptors control the level of some neuronal trace elements which are mono- and divalent cations?
9. How do the gene expressions of VP and PD alter during dehydration and chronic ethanol intoxication?

Materials and methods

Materials

Studies were carried out with adult male and female rats of the CFY, Long-Evans, Wistar and Sprague-Dawley strains, and with mice of the C57BL/6NCR and CFLP strains, using several parts of their central and peripheral nervous systems and hearts, the ileum of guinea-pigs, the lumbar stretch of the spinal cords with the descending n. ischiadicus of albino rabbits, the spinal cords of newborn pigs, the brains of carps, postmortem human brain tissues from the parietal and frontal cortex and the hippocampus from normal members of the British population (age±S.E.M.=81±8years) and from subjects with Alzheimer's disease

(age±S.E.M.=78±6years) (Newcastle General Hospital, Newcastle upon Tyne, UK), bred cells of the human neuroblastoma cell line (SH-SY5Y) which has a low passage (60-85). For the ontogenetic studies, the striatum and the cerebellum of rats from the same family but of different ages were used.

Methods

Anatomical methods: electron microscopy; enzyme histochemistry, autoradiography for light microscopical studies and histology.

Surgical procedures: electrolytic lesion; nerve ligation; nerve transection; perineural suturing; isolated ileum preparation from guinea-pig; opening of the spinal cord and the brain in order to apply substances (e.g. NVP, ibotenic acid, beta-BT and opioid substances); injection of neurotoxins (ibotenic acid and beta-BT) into the brain tissue or the ventricle in order to develop neurodegeneration; GABA microinfusion through a capillary implanted into the superior cervical ganglion.

Biochemical methods: subcellular fractionation; release of ACh by a solution with high K+ concentration; receptor solubilization; cell culturing (SH-SY5Y); measurement of enzyme activity (ChAT, AChE and BuChE) and protein content; purified membrane preparation; receptor binding.

The methods of molecular cell biology: *in situ* hybridization; *in vitro* transcription, nuclear run-on assay; Northern analysis (total RNA analysis); polymerase chain reaction; gene sequence analysis.

Experimental interventions: acute opioid treatment; chronic morphine treatment; chronic aluminum intoxication; chronic ethanol intoxication; dehydration; chronic cold stress.

Other methods: analgesia; measurement of body temperature and stereotype forms of motion.

Physical-chemical methods: atomic absorption; gas chromatography; spectrophotometry.

Mathematical methods: nonlinear regression analysis; image analysis (computer microdensitometry); statistics (ANOVA, Dunnett or Scheffe *post-hoc* tests, Student's *t*-tests).

Summary of new results and discussion

1. In the striatum, the mAChRs are already synthesized intensively during early postnatal life. The early perikaryonal synthesis of the receptors and their rapid axonal transport are responsible for the fact that the development of the striatal mAChRs precedes the development of ChAT and AChE activities. In the striatum, the B_{max} values increase in every subcellular fraction during ontogenesis, and the binding sites are highest in the microsomal and the synaptosomal fractions throughout. The number of mAChRs originating from areas outside the striatum is low. Consequently, in the subcellular fractions the measured receptor contents are due to the striatal pre- and postsynaptic receptors.

The mAChR contents of two well-defined areas of the cerebellum, the archi- and paleocerebellum, were detected during postnatal development. The distributions of these receptors in the developing cerebellum are different: in the archicerebellum, the mAChR content is higher than in the paleocerebellum, and the rate of synthesis there is faster too. Our autoradiographic studies indicated that the molecular layer of the cerebellum exhibits higher [^{3}H](-)QNB binding than the granular layer or the deep cerebellar nuclei.

The mAChRs transported in the n. ischiadicus are synthesized in the motoneurons of the spinal cord and reach their presynaptic positions by rapid migration. Our results allowed the conclusion that the mAChRs undergo two-directional (anterograde and retrograde) transport in the nerves. The anterograde transport is extensive, whereas the retrograde transport moves a much lower content of the receptors. This seems to be

proved by our autoradiographic studies. The functional role of the presynaptic mAChRs could possibly be modulation of ACh release. During neurodegenerative processes, the number of receptors transported through the axon is in direct proportion to the time of axonal regeneration; 5 months after neural transection and resuturing, the bidirectional receptor transport, which returned to 23-26% of the control value, is able to provide a basis for the function of the regenerating nerve.

2. The binding parameters of the mAChRs can be modified experimentally. We found that, as a result of *in vivo* beta-BT treatment, a nonselective, presynaptic neurodegeneration develops, which leads to decreases in both B_{max} and K_d. Repeated toxin treatment resulted in 52.2% of the control value in the case of B_{max} and 52.6% of the control in the case of Kd. During cell death, the degraded receptor content presumably has a presynaptic localization; and its deficiency might be responsible for the changes in the binding parameters. The M_2 character of the presynaptic mAChRs in the hippocampus is proved by the facts that AF-DX 116 stimulates and PZ has no effect on the release of ACh.

During cold stress, the number of hippocampal receptors increases to 166.6% of the control value, while the K_d value does not change. This change in the receptor number is remarkable since it accompanies a protein content decrease of 21.5%. It was presumed that during chronic cold stress the adaptive biochemical changes (within them the decrease in ACh content or the release can give rise to the lack of the transmitter) cause functional denervations which, through homologous regulation, stimulate mAChR synthesis.

Not all experimental interventions lead to changes in the binding parameters of the mAChRs. NVP, a specific inhibitor of ChAT, does not directly affect the mAChR content of the brain areas investigated. Although the ACh content decreases significantly in response to NVP and, can cause denervational hypersensitivity, during a short NVP treatment there is no significant *de novo* receptor synthesis. The long-lasting inhibition produced by a chronically applied GABA microinfusion into the superior cervical ganglion (although free postsynaptic thickenings of the plasma membrane appear) does not give rise to significant changes in the receptor binding parameters.

3. Binding studies on the muscarinergic radioligands ([^{3}H](+)CD, [^{3}H]PZ and [^{3}H](-)QNB) suggest that there is no significant difference between the mAChR contents in human brain tissues of patients with Alzheimer's disease and controls, but the use of [^{3}H]MCC to measure the binding of the nAChRs indicates a lower number in the disease. During our studies, we first reported the [^{3}H]PZ binding in the human frontal cortex imaged by light microscopic autoradiography, which was compared with the [^{3}H](-)QNB binding in consecutive sections. In the course of the disease, the number of somatostatin receptors decreases significantly, but there is no change in the number of delta opioid receptors. Selective lesioning of the nBM does not yield any changes either in the receptor number of the forebrain or the portion of the presumed individual receptor types. In the muscarinergic system as with other transmitter systems, we were not able to simulate faithfully all the neurochemical changes

observed in Alzheimer's disease by selective lesioning of the nBM. In the animal model in which an increased Al level characteristic of Alzheimer's disease was set up serious symptoms of cholinergic hypofunction were observed. In response to chronic Al intoxication, beside the increased endogenous Al content of the brain, significant decreases in ChAT activity and in the numbers of mAChRs and nAChRs were observed.

4. During the direct and indirect receptor binding studies of selective and nonselective muscarinergic ligands prepared in rat brain and heart tissues, human neuroblastoma cell lines and carp brain, we described mAChR heterogeneity, in part confirming the previous findings, and in part providing new results on mAChR heterogeneity. Pharmacological verification of this receptor heterogeneity was greatly promoted by our investigations and data. We first described the direct binding properties of the new cardioselective antagonist, [^{3}H]AF-DX 116 in brain and heart tissues and also in the human neuroblastoma SH-SY5Y cell line.

5. Investigations of the neuropeptide systems, their receptors, their heterogeneity and the conformational requirements of their peptide ligands led to the development of a peptide antagonist with the greatest mu opioid receptor selectivity and affinity so far. D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2, a cyclic somatostatin analogue octapeptide (CTOP), has more than 32,500-fold mu/delta selectivity, but practically no somatostatin activity. Investigations of the binding of [^{3}H]CTOP to membranes or tissue slices reveals the receptor population indicated by this radioligand to be homogeneous; selective mu opioid ligands can inhibit it with high affinity, selective delta and kappa ligands have very low inhibitory activities, and non-opioid ligands fail to inhibit at all. On the other hand, the binding of [^{3}H]DPDPE to membranes or tissue slices has high affinity towards delta receptors, and delta/mu selectivity. The specific binding can be inhibited with high affinity by using selective delta agonists or antagonists; CTOP and other mu selective ligands have low affinity to bind to these receptors.

6. In acute and chronic experiments, the physiological effects of DPDPE and CTOP were investigated through the analgesia, withdrawal effects (e.g. hypothermia and loss of body weigh) or the ability to antagonize the effects. We established that CTOP, which according to the applied test has 10-400 times greater potencies as an antagonist than naloxone, inhibits the analgesic effects of morphine effectively, in a dose dependent manner, and produces withdrawal effects rapidly in morphine-dependent animals. CTOP induces withdrawal hypothermia and loss in body weight in morphine-dependent animals. I.c.v. application of CTOP after development of a morphine-induced dependence decreases the body weight (by increasing salivation, urination and diarrhoea) in a dose dependent manner. This drug alone does not cause analgesia and does not have any effects on body weight or temperature. The fact that peripherally administered CTOP does not affect the symptoms of morphine-induced chronic dependence proves that the drug is virtually unable to cross the blood-brain barrier. This observation may be of great importance: in the development of the morphine dependence, the central and not the peripheral mu receptors play the key

role. We were among the first in the literature to publish the physiological effects of an opioid antagonist with high receptor selectivity.

In parallel with the above studies in order to elucidate the actions realized through the delta receptors, we looked into certain physiological effects of DPDPE, which has a high delta opioid receptor selectivity. Dose-dependent analgesia antagonized by naloxone was observed in mice receiving i.c.v. DPDPE. Acute tolerance developed in response to the drug, and to a lesser degree physical dependence also appeared (withdrawal hypothermia and loss in body weight). DPDPE did not end the serious symptoms of morphine withdrawal. It was concluded from our experiments that the central delta opioid receptors play a role in the processes of analgesia. In a dose-dependent manner DPDPE also increased the stereotype scratching and scenting of rats.

7. After characterizing [^{125}I]CGP 23,996, [^{3}H]DPDPE, [^{3}H]PLO17 and [^{3}H]CTOP binding to cryostat slides, we investigated the autoradiographic distribution of their specific binding in the rat brain. The distributions of the somatostatin, mu and delta receptors were examined with these conformationally restricted cyclic ligands by means of computer densitometry analysis. The autoradiographic characterization of the mu and delta opioid receptors with high selectivity and affinity was performed for the first time.

8. We observed dose- and time-dependent effects (antagonized by naloxone) of the delta opioid receptor-selective agonist DPDPE in the levels of cations and trace elements in the rat brain. A subanalgetic dose of DPDPE transiently decreased in a time- and dose-dependent manner, the levels of Ca^{2+}, Mg^{2+}, Zn^{2+} and Al^{3+} , but it did not influence the levels of Na^{+}, K^{+} and Mn^{2+}. These effects of the drug on the ions can in all cases be inhibited by naloxone pretreatment. We observed that the high Al content developed by chronic Al intoxication, which also led to a cholinergic hypofunction, can be decreased by DPDPE treatment. In the future, medical application through the delta opioid receptors could possibly decrease the endogenous Al content observed in Alzheimer's disease and probably in casual relation with it.

9. During experimental interventions, we investigated the changes in the gene expression of the neuropeptide systems in the case of VP, PD and Gs_{α} in the mouse brain. During dehydration, the VP gene expression increased in all examined hypothalamic and extrahypothalamic structures (except the n. suprachiasmaticus), and during chronic ethanol intoxication decreased in all areas. The PD gene expression also altered during these stimuli; it increased in the course of both experimental paradigms. In the areas where VP and PD can be colocalized (n. paraventricularis and n. supraopticus), these two genes produced different controls for the same stimuli, indicating that the nervous system reacts in a transmitter- and not stimulus-specific manner to the changed environment. In connection with this study, we first defined the partial sequence of the main, translating exon of the mouse PD gene, which also involves the mature hormones. During chronic ethanol intoxication, the expression of the Gs_{α} gene altered, depending on the brain areas.

Possible applications of the results

Our knowledge concerning the biochemical and receptor binding data on the mAChR types, which play an important role in the muscarinergic cholinergic transmission of the mammalian central and peripheral nervous systems, was quite defective, and thus the results reported in this thesis added further data to the previous studies, accordingly mostly having a basic research character. The realization that, in certain human diseases or as a result of some environmental stimuli, the binding parameters of the mAChRs could change, turned our attention to the importance of the receptors and the possibility of regulation by environmental factors through the receptors. The high-affinity and selectivity muscarinergic agonists and antagonists developed for the different receptor types may possible be suitable for the diagnosis or even cure of certain diseases.

With our animal models (e.g. for Alzheimer's disease or paraplegia), we were able to simulate neuropathological alterations which are almost impossible to investigate in human subjects. Although the limits of these models are obvious, they still make it possible to perform several pharmacological, biochemical and molecular biological studies.

The studies regarding the neuropeptidergic systems described in the thesis have already provided results exploitable in practice. Our studies concerning the neuropeptide receptors and the conformational requirements of their ligands resulted in the production of opioid ligands with high affinity and selectivity, and some of them can now be purchased commercially. The conformationally restricted, high-affinity ligands with selectivity for a unique receptor and high resistance against biodegradation (we participated in their development and the first tests in biological systems) are much in demand for both basic and clinical research all over the world. These drugs lack the cross-reactivity often observed in the case of the opioid receptors, which was characteristic of the previously available opioid substances and consequently they have receptor specificity. In our other studies, e.g. on the role of the delta opioid receptors in the regulation of ion movement, we followed the control of the physiological processes based on this receptor heterogeneity. Using opioid ligands in our studies, we also provided the basis for clinical research in which the aim was the restoration of the neuronal trace element content or the ion surroundings damaged during an illness or a trauma. Investigations of the gene expressions of neuropeptide systems led to the first observations of basic research, e.g. partly sequencing the main exon of the PD gene coding the mature hormones, and we increased the knowledge concerning the function of the genes in respect of molecular biology, thereby turning attention to alcoholism and the results on the level of the gene function of the development of alcohol tolerance and dependence.

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CHRONICLE

AMBRUS ÁBRAHÁM Centennial

On the occasion of the 100th anniversary of the birth of the neurologist Professor AMBRUS ÁBRAHÁM a one day celebration was organized jointly by József Attila University and the Department of Biological Sciences of the Hungarian Academy of Sciences. On this occasion, the Rector of the University announced the results of the AMBRUS ÁBRAHÁM Neurohistological Competition for Students.

The following prizes were awarded:

1st Prize: ÁRPÁD PÁLFI, ELŐD KÖRTVÉLY, KRISZTINA KOVÁCS (Albert Szent-Györgyi Medical University): Acetylcholinesterase lesions by monoclonal antibodies in the rat brain (a possible model for the cholinergic hypofunction).

2nd Prize: ÁKOS KULIN (Debrecen Medical University): Visualization of the capsaicin-sensitive dorsal root ganglionic cells and their central terminals in rat.

3rd Prize: MONIKA TARCSA (Albert Szent-Györgyi Medical University): Studies of the role(s) of amyloid in tissue cultures.

4th Prize: ILDIKÓ SCHMIDT (Debrecen Medical University): The sructure of the frog nucleus hypoglossus.

The winners presented their works on November 19, 1993. Following the lectures, the Rector of the University and the president of the Department of Biological Sciences of the Hungarian Academy of Sciences unveiled the memorial tablet to AMBRUS ÁBRAHÁM (a work by the sculpture SÁNDOR TÓTH) on the wall of the building in which Professor ÁBRAHÁM had worked for decades.

After the exhibition relating to the life of Professor ÁBRAHÁM was opened in the central building of the University, a scientific session was held to honour his scientific achievements. The following lectures were presented:

1) TIBOR FARKAS (Biological Research Center, Szeged): Remembering Professor AMBRUS ÁBRAHÁM.

2) MÁRIA CSOKNYA, ISTVÁN LENGVÁRI, LÁSZLÓ HIRIPI, KÁROLY ELEKES, MÁRTA SZEILER, JÓZSEF HÁMORI (Jannus Pannonius University, Pécs, Balaton Limnological Institute, Tihany): Changes in serotonin content during regeneration of the nervous system of *Lumbricus terrestris* L.

3) SÁNDOR BENDE, SR., SÁNDOR BENDE, JR. (Loránd Eötvös University, Budapest, Semmelweis Hospital, Miskolc): Effects of ultrastructural damage and experimental endotoxin shock in the exocrine acini of the pancreas.

4) JÓZSEF SERFŐZŐ (Kossuth Lajos University): Effects of the accumulation of xenobiotics in *Astacus leptodactylus* ESCHZ.

5) IMRE ROJIK, SÁNDOR HUSZTA, OTTÓ FEHÉR (József Attila University): Neuralgin as an early gene product.

After the scientific program, commemorative plaques were awarded to Professors SÁNDOR BENDE, SR., EMIL MINKER and JÓZSEF HÁMORI, former students of Professor ÁBRAHÁM.

The Hungarian Post Office issued a commemorative stamp for this occasion, and an autobiography of Professor ÁBRAHÁM entitled "Szálfaember" was published on this day.

The "Áron Márton" Secondary School in Csíkszereda (now in Transylvania, Romania), the former school of Professor ÁBRAHÁM, also organized a memorial day in honour of the famous scientist, and a memorial tablet was unveiled on May 6, 1994. Our University was represented by Professors GYULA FARKAS, GÉZA TURY and LÁSZLÓ KOVÁCS.

Third Hungarian Congress of Ecology

The third Hungarian Congress of Ecology, organized by the Ecological Committee of the Hungarian Academy of Sciences, The Ecological Section of the Hungarian Biological Society and the Department of Ecology, JATE University, Szeged, was held in Szeged on July 3-6, 1994.

The Congress consisted of eleven sectiones: Ecology of Plant Communities, Population Biology, Population Interactions and Behavioral Ecology, Hydrobiology, Applied and Agricultural Ecology, Nature Conservation, Theoretical Ecology and Modelling, Ecology of Animal Communities, Soil Ecology, Ecophysiology, and Ornithology. The opening plenary lecture, entitled "100 years of Hungarian Vegetation Research", was given by GÁBOR FEKETE. Altogether 300 people attended the Congress and 192 papers (talks and posters) were presented. Besides the sessions of the invited and contributed papers, Szeged, as a centre of Hungarian molecular biology research, gave an excellent opportunity to organize an informal talk with molecular biologists. Another evening program was a meeting between professional and NGO nature conservationists and ecologists.

A declaration was accepted by the plenary session in the interest of the protection of ecology against "pseudo-ecology", on the relation between the nature conservation authorities and the ecological sciences and on the necessity for the introduction of an ecologically based biomonitoring program and network in Hungary.

Besides the scientific program, the social events of the Congress were also successful, especially the get-together party, where we could enjoy the Szeged Oldtimers, a dixieland band led by an eminent ornithologist. The Congress ended with excursion to the Pusztatszer Protected Landscape area and along the River Tisza, by riverboat.

The Third Hungarian Congress was sponsored by the Hungarian Office of Nature Conservation, the Lower Tisza Regional Directorate of Water Management, the Independent Ecological Center, The Szegedért Fund and JATE University.

Awards

On 9 September 1994, the Council of JATE University conferred the title of honorary doctor on Prof. CHARLES SUSANNE, Head of Laboratory of Anthropogenetics and Ecotechnique, Vrije University, Brussels.

Prof. LAJOS FERENCZY, Corresponding Member of Hungarian Academy of Sciences, Head of the Department of Microbiology, JATE University, was awarded the Albert Szent-Györgyi Prize in acknowledgement of his scientific, scientific-organizational and lecturing work.

The council of JATE University awarded the LAJOS BARTUCZ Plaque to Dr. OTTÓ TROGMAYER, General Director of the Móra Ferenc Museum, Szeged, Head of the Department of Archeology, on the occasion of his 60th birthday.

The council of JATE University honoured Prof. PÁL LIPTÁK, Head of the Department of Anthropology between 1960 and 1980, with the title of Professor Emeritus.

Teaching on Human Biology

A course supported by TEMPUS was held in the Móra Ferenc Museum of Szeged (Hungary) on the "Teaching on Human Biology" (24-25 June, 1994). The course was organized by Prof. CHARLES SUSANNE from the Laboratory of Anthropogenetics and Ecotechnique, Vrije University Brussel, and Prof. GYULA L. FARKAS and his staff from the Department of Anthropology of József Attila University, Szeged. The lectures delivered at the program were as follows:

C. SUSANNE (Brussels): Human evolution and population genetics.

R. HAUSPIE (Brussels): Human growth modelling.

M. BUDAI and G.S. KOCSIS (Szeged): Anatomic characteristics of patients with cleft lip and palate over 3-year period.

C. PRADO (Madrid): Anthropology of Woman.

C. SUSANNE (Brussels): Nutritional aspects of growth and development.

K. CSETE, A. LÁSZLÓ, F. KÓSA, Á. VÁRKONYI and J. SZABÓ (Szeged): Untersuchung des α1-Antitrypsin(Pi) beim Neugeborenen.

GY.L. FARKAS - ZS. JUST (Szeged): Methodological notes on two cross-sectional growth studies.

C. PRADO (Madrid): Body constitution and practical applications.

B. HULANICKA (Wroclaw): Growth of children in polluted areas.

F. KÓSA (Szeged): Racial differences on the skulls of white and black human newborns.

T. SZABÓ and K. NYILAS (Nyíregyháza): Growth and development of mentally deficient children.

GY.L. FARKAS (Szeged): Influence of different factors on the age of menarche.

Participants on this course also had the opportunity to visit the Móra Ferenc Museum of Szeged and the Ópusztaszer National Memorial Park.

Habilitation proceedings

Since 1994, habilitation diplomas (decretum habilitationis) are awarded to lecturers and researchers at József Attila University who meet the prescribed requirements. Those habilitateted may bear the title habilitated doctor, abbreviated as Dr.Habil.

The University Council may award the title of University honorary lecturer to those not employed at a University if they so desire.

Habilitation may be applied for by staff who have carried out at least 5 years of prominent and creative scientific work after receiving the Candidate degree or Ph.D. or the Academic Doctor degree.

Following successful habilitation, appointment as university professor may be applied for. On 27 April 1994, two application lectures for habilitation were presented at the Biological Section of Faculty of Natural Sciences:

Dr. JÁNOS NEMCSÓK (Department of Biochemistry, JATE University): Biochemical characterization of the cholinerg system.

Dr. GÁSPÁR BÁNFALVI (Chemical-Biochemical Institute, Semmelweis Medical University, Budapest): The demonstration of structure organization of macromolecules by using models.

On 2 June, both J. NEMCSÓK and G. BÁNFALVI and those who were earlier appointed university professor (A. BARANYI, GY. FARKAS, O. FEHÉR, L. FERENCZY, F. ZSOLDOS) received their habilitation diploma from the Rector of J.A.University.

Appointments

Prof. A. BARANYI has been appointed Head of the Department of Comparative Physiology, and also elected chariman of the Biological Section in the Faculty of Natural Sciences.

INDEX